Molecular Targets for Antiepileptic Drug Development

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Summary: This review considers how recent advances in the physiology of ion channels and other potential molecular targets, in conjunction with new information on the genetics of idiopathic epilepsies, can be applied to the search for improved antiepileptic drugs (AEDs). Marketed AEDs predominantly target voltage-gated cation channels (the α subunits of voltage-gated Na^+ channels and also T-type voltage-gated Ca^{2+} channels) or influence GABA-mediated inhibition. Recently, $\alpha 2$–δ voltage-gated Ca^{2+} channel subunits and the SV2A synaptic vesicle protein have been recognized as likely targets. Genetic studies of familial idiopathic epilepsies have identified numerous genes associated with diverse epilepsy syndromes, including genes encoding Na^+ channels and $GABA_A$ receptors, which are known AED targets. A strategy based on genes associated with epilepsy in animal models and humans suggests other potential AED targets, including various voltage-gated Ca^{2+} channel subunits and auxiliary proteins, A- or M-type voltage-gated K^+ channels, and ionotropic glutamate receptors. Recent progress in ion channel research brought about by molecular cloning of the channel subunit proteins and studies in epilepsy models suggest additional targets, including G-protein-coupled receptors, such as $GABA_B$ and metabotropic glutamate receptors; hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channel subunits, responsible for hyperpolarization-activated current I_h; connexins, which make up gap junctions; and neurotransmitter transporters, particularly plasma membrane and vesicular transporters for GABA and glutamate. New information from the structural characterization of ion channels, along with better understanding of ion channel function, may allow for more selective targeting. For example, Na^+ channels underlying persistent Na^+ currents or $GABA_A$ receptor isoforms responsible for tonic (extrasynaptic) currents represent attractive targets. The growing understanding of the pathophysiology of epilepsy and the structural and functional characterization of the molecular targets provide many opportunities to create improved epilepsy therapies. **Key Words:** Epilepsy, channelopathy, antiepileptic drug, sodium channel, calcium channel, potassium channel, GABA receptor, glutamate receptor, GABA transporter, glutamate transporter, gap junction.

INTRODUCTION

Our understanding of the pathophysiology of the epilepsies has advanced dramatically in the last 30 years, especially in terms of their cellular physiology and genetics. Drug treatment of epilepsy has also made remarkable strides, with the introduction of 11 new antiepileptic drugs (AEDs) since 1978: valproate, vigabatrin, tiagabine, lamotrigine, oxcarbazepine, felbamate, topiramate, gabapentin, levetiracetam, zonisamide, and pregabalin. Improvement in terms of clinical outcome, however, has fallen short of expectations, with up to one third of patients continuing to experience seizures or unacceptable medication-related side effects in spite of efforts to identify optimal treatment regimes with one or more drugs.[1,2]

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Since the landmark identification of the anticonvulsant properties of phenytoin in 1936 by virtue of its ability to protect against electroshock-induced convulsions in the cat,[3,4] the majority of novel AEDs have been identified through screening in animal models of epilepsy. The most widely used *in vivo* models have been the maximal electroshock (MES) test in normal mice and rats and the pentylenetetrazol (PTZ) test in normal mice.[5] In the 1970s, understanding of the role of GABA as an inhibitory neurotransmitter in the brain led to the design of compounds that would modify the disposition of GABA so as to increase its inhibitory activity and thereby protect against seizures. This approach led to vigabatrin,[6] which increases cellular GABA by inhibiting GABA-transaminase,[7] and to tiagabine,[8] which increases synaptic GABA levels by competitively inhibiting the GABA reuptake transporter GAT-1.[9]

To this day, vigabatrin and tiagabine are the only

rationally designed AEDs. Other marketed AEDs have been identified by empirical screening in animal models. It has been argued that the latter approach, which is unbiased with respect to mechanism, provides an opportunity to uncover drugs that act in new ways and through new targets.[10] Although empirical screening continues to be valuable, this review considers other strategies to identify new AEDs, with an emphasis on applying new information from epilepsy genetics and structural and functional studies of molecularly cloned ion channels and other targets.

NEW APPROACHES TO IDENTIFY AED MOLECULAR TARGETS

Empirical screening has successfully led to the identification of many useful AEDs, each with unique properties and distinct clinical profiles.[11] Nonetheless, the observation that many epilepsy patients do not experience adequate seizure control with currently available drugs raises the possibility that existing screening methods may bias toward a restricted set of molecular targets and mechanisms. Are there approaches capable of defining specific molecular targets that could be used to identify molecules with antiseizure activity that is distinct from that of established agents? We consider three such approaches.

The first approach is to consider the molecular targets of natural or synthetic proconvulsant agents. A vast amount of data on seizure-precipitating substances has been accumulated over the last century.[12] These data point to $GABA_A$ receptors specifically and to GABA mechanisms generally as key targets of convulsants.[13] Thus, seizures occur in animals and humans if GABA synthesis is impaired (as in pyridoxine deficiency or by exposure to isoniazid, a pyridoxine antagonist) or if $GABA_A$ receptors are inhibited by any one of a diverse group of compounds such as bicuculline, PTZ, picrotoxin, penicillin, convulsant barbiturates, and inverse benzodiazepine-site ligands. Toxins that slow inactivation of voltage-gated Na^+ channels and broaden action potentials, such as scorpion α-toxins, also are convulsant.[14–16] In addition, the activator of voltage-activated Ca^{2+} channels Bay K 8644 is a potent convulsant.[17] Voltage-activated Na^+ and Ca^{2+} channels and $GABA_A$ receptors represent the major targets of AEDs.[11] Seizures are elicited when the major anticonvulsant targets are pharmacologically activated in a manner that is functionally opposite to the therapeutic mode of action of the AEDs. (In the case of voltage-activated Ca^{2+} channels, it is L-type channels that are activated by Bay K 8644, whereas T-type and possibly other non-L-type Ca^{2+} channels are the anticonvulsant targets.) Ionotropic glutamate receptors are also identified as potential AED targets by this approach, because agonists of both NMDA- and AMPA-type glutamate receptors can elicit seizures.[18,19] It is certainly the case that drugs that block ionotropic glutamate receptors have anticonvulsant properties, at least in animal models.[20] Therefore, it would appear that AED targets correspond to the sites of action of convulsant substances.

Are there other potential convulsant targets that might be applied to the identification of AEDs? Blockers of voltage-gated K^+ channels are powerful convulsants. Such agents include antagonists of delayed rectifier and A-type channels such as 4-aminopyridine, dendrotoxin I, tityustoxin-Kα, and pandinustoxin-Kα.[21–23] In addition, linopirdine, a blocker of M-type (KCNQ) K^+ channels, also has convulsant actions, at least in the immature brain.[24] To date, no openers of delayed rectifier/A-type K^+ channels have been identified. However, retigabine and newer KCNQ K^+ channel openers have anticonvulsant activity in animal models and retigabine has shown clinical efficacy.[25] Finally, many metabolic poisons induce seizures. Studying the pathophysiology of such seizures may be useful to understanding the therapeutic action of the ketogenic diet, which could define an entirely new set of targets for AEDs.[26]

The second novel approach to identifying AED targets is to select from among the cellular elements that have a physiological role in the generation of rhythmic discharges and epileptic phenomena in model preparations. This approach identifies all of the ligand-gated and voltage-gated ion channel mentioned above, including $GABA_A$ and glutamate receptors and voltage-activated Na^+ and Ca^{2+} channels. In addition, however, it suggests some unexpected targets. Thus, progressive understanding of the role of gap junctions in neuronal synchronization and in the generation of epileptic discharges has led to the identification of connexins as potential targets.[27]

Similarly, studies showing changes associated with epileptogenesis in hyperpolarization activated cation channels mediating I_h have raised the possibility that these channels could be an appropriate target.[28] Molecules involved in the regulation (for example, by phosphorylation) or trafficking of ion channels implicated in epileptic phenomena are also potential AED targets. Pharmacological agents that can influence these processes are not generally available to evaluate this hypothesis. There is, however, evidence that topiramate may act indirectly on ion channels through regulation of their phosphorylation state.[29] Neurotrophins and their receptors are other potential targets. For example, brain-derived neurotrophic factor (BDNF) can modulate the functional properties of ion channels through TrkB-mediated activation of intracellular second messenger cascades and protein phosphorylation, and may also directly and rapidly gate ion channels.[30] In addition, BDNF has been implicated in synaptic plasticity and epileptogen-

TABLE 1. *Voltage-Gated Na^+ Channels: Genes, Epilepsy Syndromes, and Pharmacology*

Gene	Channel	Na^+ Current	Epilepsy Syndromes	Pharmacology	AED Actions
SCN1A	$Na_v1.1$	Transient	GEFS+ type 2, SMEI, ICEGTCS	Tetrodotoxin, local anesthetics (*block action potentials*)	Phenytoin, carbamazepine, lamotrigine; possibly topiramate *(stabilize inactivation)*
SCN2A	$Na_v1.2$	Transient	GEFS+ type 2, BFNIS	Tetrodotoxin, local anesthetics (*block action potentials*)	Phenytoin,[47,48] carbamazepine, oxcarbazepine, lamotrigine,[49,50] zonisamide; possibly felbamate, topiramate, and valproate (*stabilize inactivation*)
SCN1B			GEFS+ type 1		
SCN8A	$Na_v1.6$	Transient and persistent		Riluzole (*decreases persistent*)	Phenytoin, topiramate, valproate (*decrease persistent*)

Where reference citations are provided, AED action has been confirmed with recombinant, expressed channels.
BFNIS = benign familial neonatal–infantile seizures[43]; GEFS+ = generalized epilepsy with febrile seizures plus (types 1 and 2); ICEGTCS = intractable childhood epilepsy with generalized tonic clonic seizures; SMEI = severe myoclonic epilepsy of infancy.

esis,[31] suggesting that it could be a target for antiepileptogenic therapies.

The third novel approach to defining AED targets is to consider as candidates the protein products of genes associated with epilepsy syndromes in animals and in humans. The genes for most Mendelian human epilepsies have now been identified, and in many cases the way in which mutations in the gene product alter function has been established (although how these functional changes lead to the epileptic phenotypic is sometimes obscure). The genes associated with epilepsy syndromes encode predominantly ion channels[32,33] (TABLES 1–3), which, remarkably, are in many cases the same ion channels that are targeted by drugs discovered through empirical screening or that would have been identified through the "convulsant target" or the "physiological target" methods. Consequently, voltage-gated and ligand-gated ion channels are the preeminent targets for AEDs. Indeed, when linkage studies have identified genes of unknown function as candidate epilepsy genes, the gene products have often turned out to be ion channel subunits or proteins associated in some way with ion channels (see discussion of EFHC1[34] and LGI1[35] in the sections on voltage-gated calcium and potassium channels, respectively).

It has been estimated that there are only about 300 human ion channel genes, so the universe of potential targets is significantly narrowed from the 20,000–25,000 protein coding genes contained in the human genome.[36] Indeed, the pharmaceutical industry now has the capability of screening against all known ion channel genes. Despite their clear importance, however, ion channels are not the only potential AED targets, and in this review we also consider relevant transporters, enzymes, and second messenger systems.

VOLTAGE-GATED ION CHANNELS

Neuronal excitability is determined by the properties of the ion channels in the neuronal membrane such that the aberrant excitability associated with an epileptic discharge will necessarily be mediated by voltage-gated and ligand-gated ion channels, and may even be the result of defects in the function of these channels. Because of the pivotal role played by these ion channels in the physiology of all forms of epilepsy, they are obvious AED targets and it is perhaps no surprise that several of these channels represent the critical sites of action for AEDs that were originally identified by empirical screening in animal models.

Voltage-gated ion channels are now seen as forming a superfamily (referred to as the "voltage-gated-like ion channels") comprising 143 genes.[37,38] This superfamily encompasses the S4 family in which the pore-forming subunits are built on a motif of six transmembrane segments (S1–S6) the fourth of which (S4) contains a voltage-sensing element. In voltage-gated Na^+ and Ca^{2+} channels, four such domains (referred to as I–IV or D1–D4) are expressed as a single polypeptide arranged around a central pore that conducts the ionic current. In voltage-gated K^+ channels, the channel is a tetramer of four individual subunits, each of which contains a single S1–S6 domain. In all of the voltage-gated channels, four re-entrant pore loops (P-loops) between the S5 and S6 segments form the external mouth of the ion channel. A highly conserved sequence within this loop confers the

TABLE 2. *Voltage-Gated Ca^{2+} Channels: Genes, Epilepsy Syndromes, and Pharmacology*

Gene*	Channel Subunit	Ca^{2+} Current	Epilepsy Syndrome		Pharmacology	AED Actions
			Mouse	Human		
CACNA1S	$Ca_v1.1$	L-type (HVA)			Dihydropyridines (*block*); Bay K 8644 (*activates*)	? Barbiturates, ? felbamate (*decrease*)
CACNA1C	$Ca_v1.2$					
CACNA1D	$Ca_v1.3$					
CACNA1F	$Ca_v1.4$					
CACNA1A	$Ca_v2.1$	P/Q-type (HVA)	*tottering*, *rocker*, *leaner*, *roller*	AEA, EAT2+E	ω-Agatoxin IVA (*blocks*)	Lamotrigine, ? oxcarbazepine, ? levetiracetam (*decrease*)
CACNA1B	$Ca_v2.2$	N-type (HVA)			ω-Conotoxin GVIA (*blocks*)	Lamotrigine, ? oxcarbazepine (*decreases*); gabapentin, pregabalin (*bind*)
CACNA1E	$Ca_v2.3$	R-type (HVA)				
CACNA1G	$Ca_v3.1$	T-type (LVA)				Ethosuximide,[68] zonisamide *(decrease)*
CACNA1H	$Ca_v3.2$					
CACNA1I	$Ca_v3.3$					
CACNA2D1	$\alpha2$–δ subunit		*ducky*			
CACNA2D2						
CACNB1–4	β subunit		*lethargic* ($\beta4$)			
CACNG1–7	γ subunit		*stargazer* ($\gamma2$)			
EFHC1†	Interacts with $Ca_v2.3$			JME		
SNAP25	Interacts with HVA (P/Q-type)	Increases LVA	*coloboma*			

AEA = absence epilepsy with ataxia; CAE = childhood absence epilepsy; EAT2+E = episodic ataxia type 2 with epilepsy; FHPM = familial hemiplegic migraine; HVA = high voltage activated; JME = juvenile myoclonic epilepsy; LVA = low voltage activated. *Human gene designations; mouse orthologs use lower case (with an initial capital letter). †*EFHC1* is homologous to a *Chlamydomonas* axonemal protein, Rib72, and its mouse ortholog, mRib72-1/Efhc1, which is expressed somatodendritically in neurons and is colocalized with $Ca_v2.3$.

specificity for the conducted ion (Na^+, Ca^{2+} or K^+). The tetrameric organization of voltage-gated K^+ channels is also present in calcium-activated K^+ channels and cyclic nucleotide-gated and the hyperpolarization-activated cyclic nucleotide-modulated cation channels.

Voltage-gated-like ion channels not only control excitability in the central nervous system, but also in the peripheral autonomic nervous system, the cardiovascular system, and the digestive system. They also control all secretory functions including the release of hormones. Thus, in selecting an ion channel as a molecular target, it is necessary to be cognizant of which tissues express the channel. Importantly, the voltage-gated Na^+, Ca^{2+} and K^+ channels expressed in the heart are often distinct from, but closely homologous to, those expressed in the brain. Drugs need to be selected that preferentially select for the isoforms in the brain over those in the heart.

Voltage-gated sodium channels

The mammalian voltage-gated Na^+ channels that have been functionally expressed so far form a single family of nine genes encoding the pore-forming α subunits.[39] Four of these are expressed predominantly in the central nervous system—$Na_v1.1$ (*SCN1A*), $Na_v1.2$ (*SCN2A*), $Na_v1.3$ (*SCN3A*), and $Na_v1.6$ (*SCN8A*)—and two in the peripheral nervous system and dorsal root ganglia—$Na_v1.7$ (*SCN9A*) and $Na_v1.8$ (*SCN10A*). $Na_v1.4$ (*SCN4A*) is expressed in skeletal muscle. $Na_v1.5$ (*SCN5A*) is expressed principally in cardiac muscle, but is also found in some limbic neurons in the rat brain, including the piriform cortex. $Na_v1.3$ is significantly expressed in the brain only early in development. $Na_v1.9$ (*SCN11A*) is expressed widely in the brain and spinal cord and contributes to the persistent, tetrodotoxin-resistant sodium current in small-diameter dorsal root ganglion neurons. It

TABLE 3. *Voltage-Gated K^+ Channels: Genes, Epilepsy Syndromes, and Pharmacology*

Gene*	Channel Subunit	K^+ Current	Epilepsy Syndrome		Convulsant Pharmacology	AED Actions
			Mouse	Human		
KCNA1	$K_v1.1$	Delayed rectifier, A-current	Knockout → seizures	EAT1-MK-PS	4-AP, dendrotoxin, tityustoxin-Kα, pandinustoxin-Kα (*block*)	
LGI1	Auxiliary subunit for $K_v1.1$; coassembles with $K_v1.4$, $K_v\beta$	A-current in axon terminals, especially temporal cortex		ADLTLE		
KCNAB2	$K_v\beta2$ assembles with K_v1	May modulate delayed rectifier, A-current	Knockout → seizures			
KCNQ2	$K_v7.2$	M-current, slow K	Knockout → seizures	BFNC	Linopirdine, XE991 (*block*)	Retigabine, ICA-27243 (*open*)
KCNQ3	$K_v7.3$					
KCNJ3	$K_{ir}3.1$	Girk1		Absence		
KCNJ6	$K_{ir}3.2$	Girk2 (ATP-sensitive)	*weaver*; Knockout → seizures			
KCNJ10	$K_{ir}4.1$		Seizure susceptibility	Genetic association but no functional effects of mutations		
KCNJ11	$K_{ir}6.2$	ATP-sensitive	Knockout → seizure susceptible after hypoxia	Tonic–clonic seizures		
KCNMA1	$K_{Ca}1.1$	BK Ca^{2+}-activated K^+ current (slo1)		GEPD		
KCNK9	TASK3	TWIK-like acid-sensitive background current	GAERS	Childhood absence		

4-AP = 4-aminopyridine; ADLTLE = autosomal dominant lateral temporal lobe epilepsy with auditory features; BFNC = benign familial neonatal convulsions; EAT1-MK-PS = episodic ataxia type 1 with myokymia and partial seizures; GAERS = genetic absence epilepsy rats from Strasbourg; GEPD = generalized epilepsy with paroxysmal dyskinesia. *Human gene designations; mouse orthologs use lower case (with an initial capital letter).

is also expressed in peripheral tissues including the spleen, small intestine, and placenta.

In the mammalian brain, the fast, transient Na^+ currents that generate action potentials are mediated by $Na_v1.1$ and $Na_v1.2$ isoforms (predominant in the rostral brain, globus pallidus, hippocampus, thalamus, and cerebellum) and by the $Na_v1.6$ isoform (prominent in the somatodendritic regions of output neurons of the cerebellum, cortex and hippocampus). These channels also mediate the persistent, resurgent, or late Na^+ current, which (although much smaller than the fast inactivating Na^+ current) may play a significant role in epilepsy and in the action of AEDs.[11]

The persistent current is greater for $Na_v1.6$ than for $Na_v1.1$ or $Na_v1.2$. Persistent Na^+ currents are thought to contribute to burst discharges in the hippocampus after pilocarpine-induced status epilepticus by enhancing after-depolarizing potentials. There are four auxiliary (β) subunits ($Na_v\beta1$–$Na_v\beta4$; genes *SCN1B–SCN4B*) that have an intramembrane segment and an immunoglobulin-like extracellular element. All four can be found in association with the α-units expressed in the brain.

Mutations in the α subunits have been found in various clinical syndromes, including the long QT syndrome, periodic paralysis, paramyotonia congenita, and various forms of idiopathic generalized epilepsy, including gen-

eralized epilepsy with febrile seizures plus (GEFS+) (TABLE 1). The first mutation identified in a family with GEFS+ was in *SCN1B* (C121W). This mutation, when expressed in oocytes, depresses fast inactivation and increases the Na^+ current through loss of the modulatory function of the β subunit.[40] Subsequently, more than 100 different mutations in *SCN1A* and *SCN2A* were identified in GEFS+ families, including some in the S4 voltage sensor. The functional consequences of these mutations in expression systems have proven to be diverse, and not simply related to enhanced excitability, although some enhance persistent current.[41] Computer simulations, however, suggest that the changes can explain increased neuronal firing.[42]

Two other severe syndromes of early life are associated with mutations in *SCN1A*. Severe myoclonic epilepsy of infancy (SMEI) manifests many seizure types often evolving into a malignant syndrome. A large number of mutations, some occurring de novo and some involving the ion pore, have been identified in *SCN1A*.[43,44] Intractable childhood epilepsy with generalized tonic–clonic seizures (ICEGTCS), also due to mutations in *SCN1A*, is similar clinically to SMEI except that there is reduced severity of psychomotor impairment and no myoclonus.[45] Mutations in *SCN2A* have recently been associated with benign familial neonatal–infantile seizures (BFNIS)[46]; the functional effects of these mutations are not known.

Studies of expressed α subunits with single site mutations have demonstrated that local anesthetics, antiarrythmics, and Na^+ channel-blocking AEDs act on overlapping sites on the α subunit of voltage-gated Na^+ channels.[47–50] The different clinical actions of the drugs can be explained in terms of specific effects on channel function. AEDs that modulate voltage-dependent Na^+ channels produce a voltage- and use-dependent block of the channels by binding predominantly to the inactivated state of the channels, thus suppressing high-frequency, repetitive action potential firing. The critical downstream effect may be to reduce action potential–dependent synaptic neurotransmitter release during the high-frequency firing that occurs with epileptic discharges.[51,52]

Note that glutamate release is selectively inhibited with less effect on the release of GABA.[53,54] One group has reported that phenytoin and lamotrigine actually increase the spontaneous release of GABA, which they have proposed as contributing to the anticonvulsant actions of the drugs.[55,56] *In vivo* microdialysis studies have confirmed that carbamazepine, oxcarbazepine, and lamotrigine inhibit glutamate release, but those studies are not entirely consistent with the view that this constitutes the primary anticonvulsant mechanism.[57] Voltage-dependent Na^+ channel block may also reduce the propagation of action potentials from the soma into the dendrites[58,59] and the dendritic amplification of synaptic potentials.[60]

Localization of the precise site of AED action on the $Na_v1.2$ channel was provided by studies of the effects of single site-directed mutations in expressed recombinant α subunits. The binding of phenytoin and the frequency-dependent block of Na^+ current was diminished by mutations F1764A and Y1771A in transmembrane segment IVS6.[47,48] Further studies with lamotrigine and some related compounds revealed that the nearby I1760 and additional residues in IIIS6 are also critical to drug binding[49,50,61] (FIG. 1). During gating, the structure of the Na^+ channel changes such that the critical amino acid residues in IIIS6 and IVS6 become exposed to the pore in the activated and inactivated states. This enables docking of lamotrigine within the pore in a use-dependent fashion.

Why do different Na^+ channel-blocking AEDs have distinct clinical profiles? There are at least three possibilities. First, the biophysical parameters for Na^+ channel block may differ in terms of binding kinetics and selectivity for the different gating states of the channel. In fact, there are marked differences in these parameters among the classical Na^+ channel-blocking AEDs phenytoin, carbamazepine and lamotrigine, which could, in part, account for their distinctive clinical properties.[62,63] Recently, there has been some success in attempts to optimize the biophysical parameters of Na^+ channel blocking drugs. For example, the semicarbazone anticonvulsant V102862 (Co 102862), discovered by empirical screening in animal models, was found to block Na^+ channels. Analogs with enhanced Na^+ channel blocking potency were discovered using an electrophysiological screen.[64] Recent biophysical studies of one of these analogs, PPPA {2-[4-(4-chloro-2-fluorophenoxy)phenyl]-pyrimidine-4-carboxamide}, demonstrated enhanced state-dependence (selectivity for inactivated *versus* resting channels) compared to carbamazepine and lamotrigine, and, correspondingly, a greater protective index (comparing potency for rotarod toxicity with activity in the MES test).[65] Thus, it may be possible to identify compounds with potentially improved clinical activity based on a detailed characterization of their biophysical parameters for block of Na^+ channels.

A second way in which Na^+ channel blockers can differ is in their selectivity for Na^+ channel isoforms. $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ have very different patterns of expression in neurons and among brain regions, so that selective targeting might protect against seizures with reduced side effects compared with nonselective antagonists. Selective targeting of isoforms, such as $Na_v1.6$, that contribute more to persistent current than other isoforms might also optimize anticonvulsant activity. Designing drugs to achieve such selectivity among paralogs may be challenging, but the examples provided

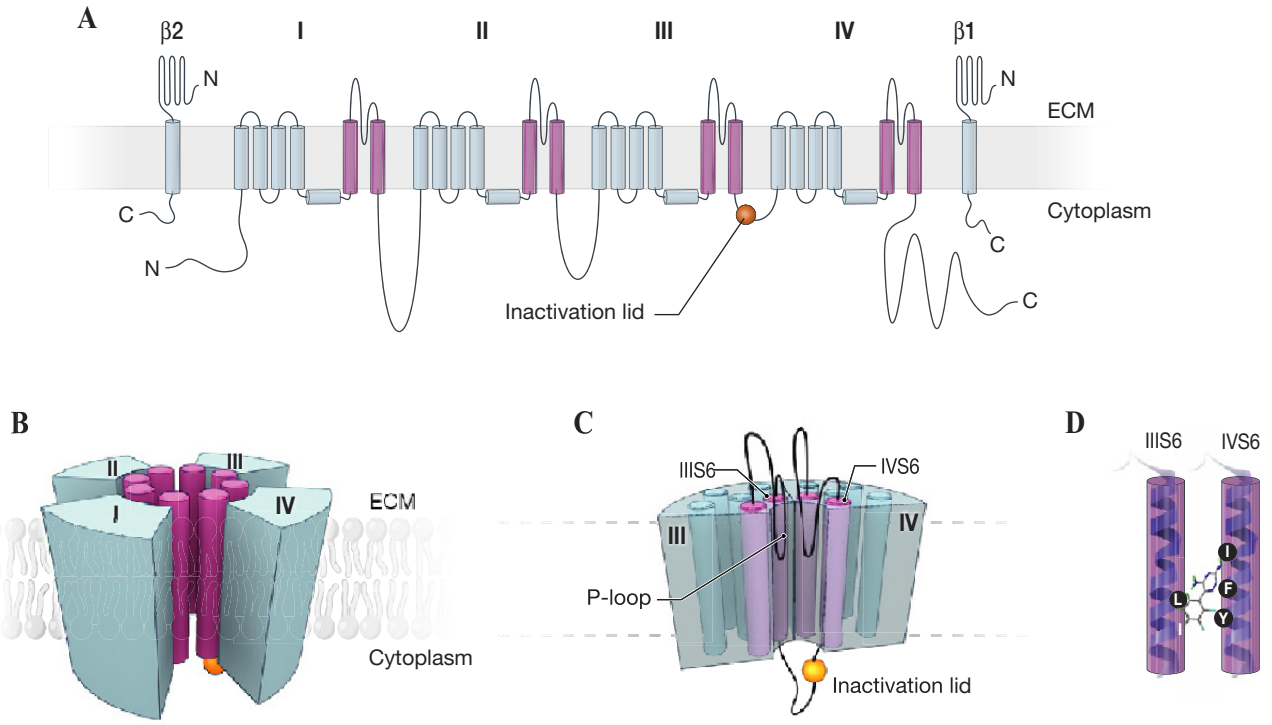

FIG. 1. Voltage-gated Na^+ channels. **A:** Membrane topology of the α subunit of a voltage-gated Na^+ channel and auxiliary β1 and β2 subunits. Note that the four homologous domains of the α subunit (labeled I to IV) are formed from one contiguous peptide in which each domain is analogous to an α subunit of a voltage-gated K^+ channel (FIG. 3A). **B:** Perspective diagram showing how the S5–S6 transmembrane elements form, with their P-loops, a ring surrounding the ion-selective pore. **C:** Schematic diagram of domains III and IV showing the gating hinge or "inactivation lid" that connects them. **D:** Diagram showing the critical residues that determine the binding of lamotrigine to pore domains III (L1465, I1469) and IV (I1760, F17654, Y1771), as shown by mutagenesis studies.[40,41] Part D is adapted from Lossin et al.,[41] with permission.

by tetrodotoxin and pyrethroid insecticides indicate that it is achievable. Thus, tetrodotoxin shows exquisite selectivity, blocking Na^+ channel isoforms with an aromatic residue in the first intracellular linker (phenylalanine 383 in $Na_v1.1$, tyrosine 401 in $Na_v1.4$, cysteine 374 in $Na_v1.5$), but not channels in which a polar residue is present at this site (as in $Na_v1.8$ and $Na_v1.9$). Similarly, pyrethroid sensitivity can be altered by single amino acid substitutions.

Na^+ channels are present at distinct sites in neurons, where they subserve different functions and play distinct roles in epileptic discharges. Thus, selective targeting of Na^+ channels isoforms expressed in different parts of the neuron may produce markedly different functional effects. Some Na^+ channels, especially those at the initial axon segment, initiate action potentials and control firing thresholds and the thresholds of burst discharges. Postsynaptic somatodendritic Na^+ channels act in concert with a range of ligand-gated and voltage-gated channels to generate interictal and ictal neuronal discharges. In contrast, presynaptic Na^+ channels contribute to the regulation of neurotransmitter release. All Na^+ channel blocking AEDs will act to some extent on these three Na^+ channel populations, but there may be differences in the relative effects on one population. For example, some Na^+ channel blocking AEDs may preferentially inhibit glutamate release as a result of selective interactions with Na^+ channels that are located on presynaptic glutamatergic terminals.[53]

The third way in which Na^+ channel blocking AEDs may differ is in their secondary effects on other voltage-gated or ligand-gated ion channels. Several marketed AEDs that are known to block Na^+ channels (including felbamate, topiramate, and zonisamide) also have prominent interactions with other ion channel targets. The combination of actions may contribute to the unique clinical efficacies of each of these drugs.

In addition to explaining differences in the clinical spectrum of action among currently available Na^+ channel blocking AEDs, these principles suggest that it may be possible to optimize the activity of drugs that target Na^+ channels, with the prospect that new compounds may have improved activity or reduced side effects.

Voltage-gated calcium channels

Like other voltage-gated ion channels, voltage-gated Ca^{2+} channels contribute to the membrane potential behavior of neurons; they are particularly important in rhythm generation and burst firing. Because Ca^{2+} serves

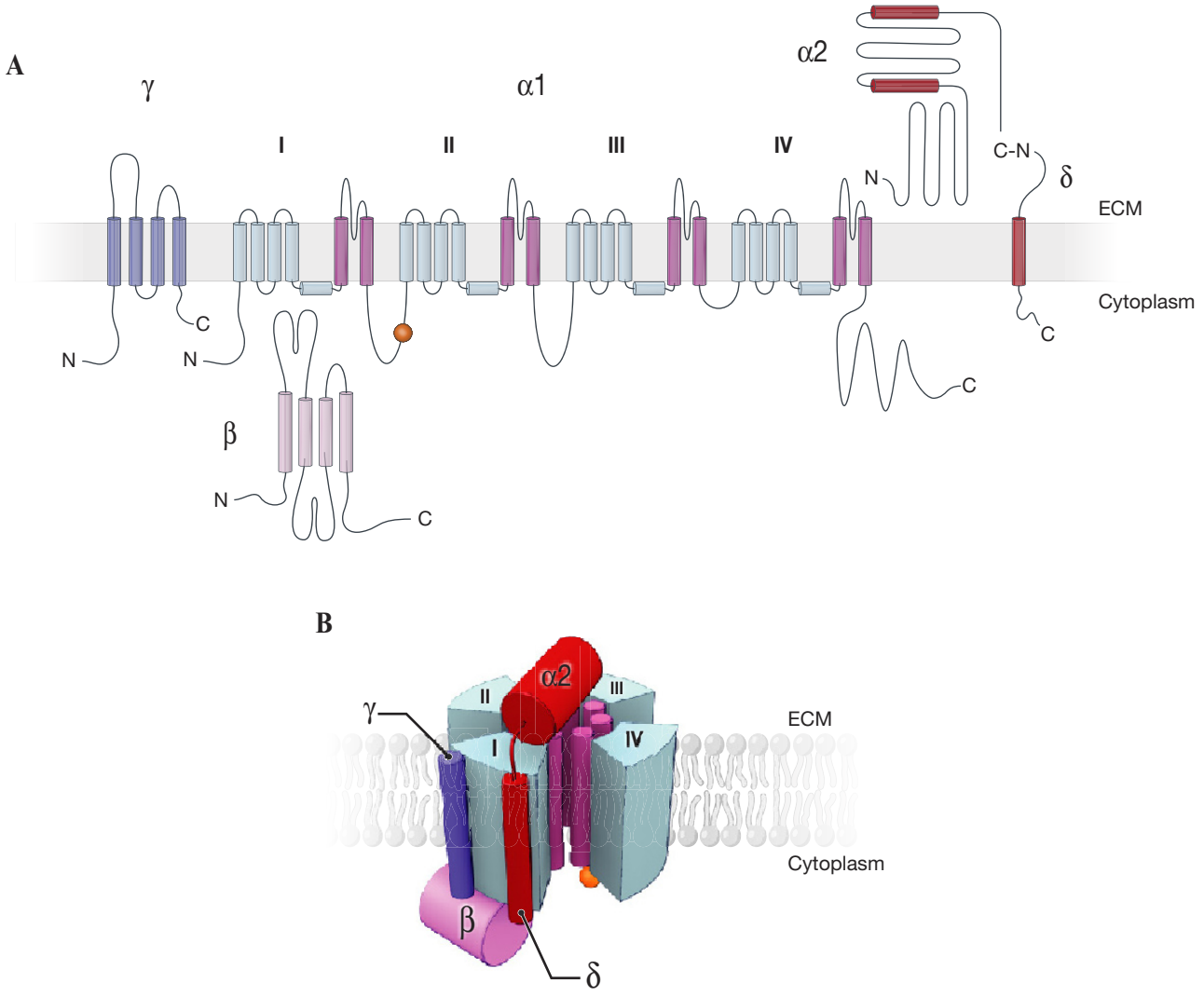

FIG. 2. Voltage-gated Ca^{2+} channels. **A:** Membrane topology diagram of the α_1 and the α_2 δ, β, and γ subunits of a voltage-gated Ca^{2+} channel. The β subunit is intracellular and binds to the loop connecting domains I and II. **B:** Perspective diagram showing the α_1 subunit forming the ion-selective pore with its four surrounding voltage-sensing domains and the auxiliary subunits. Part B is adapted from Wolf et al.,[68] with permission.

as a messenger, Ca^{2+} channels have the unique ability among ion channels to couple membrane electrical events to diverse cellular functions, including exocytosis of neurotransmitters.

The pore-forming α_1 subunits of the voltage-gated Ca^{2+} channels are homologous in structure to the α subunits of voltage-gated Na^{+} channels (FIG. 2). They fall into three subfamilies, corresponding to channel types earlier classified according to their biophysical and pharmacological properties: L-type (high voltage-activated, generating a long-lasting current); N, P/Q, and R-types (expressed in nerve terminals and responsible for the Ca^{2+} entry that triggers neurotransmitter release); and T-type (low voltage-threshold, generating a transient current, somatodendritic localization, and critical to pacemaker activity and some patterns of burst firing).[66,67] The α_1 subunits are variably associated with auxiliary subunits, including the intracellular β subunits (β1–β4), the largely intramembranal γ subunits (γ1–γ8), and the intramembranal/extracellular α2–δ subunits (types 1–4)[68] (FIG. 2).

A surprising variety of mutations involving voltage-gated Ca^{2+} channels have been identified in mice that exhibit absence-like seizures with 5- to 7-Hz spike-and-wave cortical discharges[69–71] (TABLE 2). These are all recessive syndromes and involve several different specific Ca^{2+} channel subunit types, but not L-type or T-type channels. L-type channels have not been associated with epilepsy syndromes in mice or humans and are not considered to be targets for AEDs. T-type Ca^{2+} channels are believed to be the targets of antiabsence agents such as ethosuximide, which weakly block native and recom-

binant T-type Ca^{2+} channel currents.[72–74] As expected from this pharmacological observation, mice that lack α_{1G} T-type Ca^{2+} channels are resistant to absence seizures.[75,76]

The three recessive mutations in *Cacna1a* ($Ca_v2.1$) that produce absence-like syndromes in *tottering*, *leaner* and *rocker* mice all impair channel function, reducing P/Q-type Ca^{2+} currents. These and *rolling Nagoya* mice (with a further mutation on *Cacna1a* but not showing absence seizures) show ataxia and a wide variety of other central nervous system changes. The *stargazer* mouse has a mutation in *Cacng2* that alters the function of $Ca_v2.1$ and $Ca_v2.2$[77] and modifies the cell surface expression of AMPA receptors.[78] The mutation in the *lethargic* mouse interferes with the β_4 subunit modulatory action on the α_1 subunit $Ca_v2.1$ and $Ca_v2.2$ although some compensatory subunit insertions ("reshuffling") may occur.[79] In *ducky* mice, the gene for the $\alpha2$–δ-2 subunit is mutated, and this also alters the function of the $Ca_v2.1/2.2$ channels.[80] Targeted disruption of the *Cacna2d2* gene leads to ataxia and enhanced seizure susceptibility.[81] In sum, a common factor in these six very different mouse syndromes expressing absence-like seizures is impaired function in presynaptic Ca^{2+} channels (P/Q-type) controlling neurotransmitter release. Proteins involved in the SNARE complex that link P/Q-type Ca^{2+} channels to synaptic vesicle release (SNAP-25, syntaxin, synaptotagmin)[82] play an essential role in neurotransmitter release. Note that *coloboma* mouse, which bears an autosomal dominant mutation affecting the SNAP25 protein, exhibits spike-and-wave discharges.[83]

Only recently have mutations in voltage-activated Ca^{2+} channel subunits been associated with human epilepsy syndromes. A syndrome of absence epilepsy with episodic ataxia similar to that observed in mice has been described in a family with a mutation in the α_{1A} subunit ($Ca_v2.1$).[84] In childhood absence epilepsy (CAE), 12 mutations involving *CACNA1H* (encoding the α_1 subunit $Ca_v3.2$) have been reported.[85,86] Functional expression studies with several of these mutations have revealed a gain of function.[87,88] At least 30 mutations in *CACNA1H*, some involving splicing defects, are associated with CAE and other idiopathic generalized epilepsies.[89] Five mutations in *EFHC1*, a gene encoding a protein with an EF-hand motif, have been found in families with juvenile myoclonic epilepsy (JME).[34] This protein associates with the R-type Ca^{2+} channel $Ca_v2.3$. EFHC1 increases R-type Ca^{2+} currents, but this activity is lost when the protein bears the mutations associated with JME. A tentative association between polymorphisms in a further EF-hand containing gene (*EFHC2*) has also been found in families with JME.[90]

Many AEDs have been reported to inhibit Ca^{2+} currents (TABLE 2). Only in the case of agents such as ethosuximide, which act on T-type Ca^{2+} channels, is there firm evidence that Ca^{2+} channels are the primary target for seizure protection. The anticonvulsant action of the barbiturate phenobarbital may, however, be due, at least in part, to inhibition of Ca^{2+} current, as well as an action on $GABA_A$ receptors.[91] Similarly, although the anticonvulsant activity of lamotrigine is believed to be mediated primarily by effects on voltage-gated Na^+ channels, lamotrigine also inhibits high voltage-activated (N- and P/Q-type) Ca^{2+} channels (but inhibits R-type minimally, and T- or L-type Ca^{2+} channels not at all).[92–95] Whereas effects on Na^+ channels are believed to be responsible for the inhibitory action of Na^+ channel-blocking AEDs on synaptic glutamate release, inhibitory actions on voltage-gated Ca^{2+} channels may also contribute, especially for lamotrigine (through its blocking action on N-type channels).[96,97]

Recently, it has become apparent that the molecular targets for gabapentin and pregabalin are $\alpha2$–δ proteins, specifically $\alpha2$–δ-1 and $\alpha2$–δ-2.[98,99] The evidence supporting this concept has been reviewed.[55] At present, the exact way in which binding to these proteins protects against seizures is not fully understood. Some investigators (but not all) have observed inhibitory effects on voltage-gated Ca^{2+} currents and have reported that these effects can be partially occluded by toxins that selectively block either P/Q- or N-type Ca^{2+} channels.[100–102] Other studies have shown inhibition of the release of glutamate and other neurotransmitters.[103–105]

The variability in the effects on Ca^{2+} current may relate to differences in expression of the $\alpha2$–δ subunit in different cell types or in response to different conditions. For example, in chronic neuropathic pain states, $\alpha2$–δ subunits may be upregulated, conferring gabapentin and pregabalin sensitivity.[106,107] Whether there are similar plastic changes in $\alpha2$–δ subunit expression in epilepsy remains to be determined. Although gabapentin and pregabalin inhibit neurotransmitter release in many systems, there is evidence that this may not require inhibition of calcium influx and may instead be mediated by an interaction of $\alpha2$–δ (or the calcium-channel complex containing $\alpha2$–δ) with synaptic proteins that are involved in the release or trafficking of synaptic vesicles.

Voltage-gated potassium channels

The most diverse group of ion channels, K^+ channels serve to limit excitability in neural cells. They are formed from α subunits, which comprise the ion-conduction pore, selectivity filter, and gating apparatus, and various auxiliary subunits that serve modulatory roles. The typical K^+ channel is a tetramer of individual α subunits, each one of which is homologous to a domain of the pore-forming α or α_1 subunits of voltage-gated Na^+ or Ca^{2+} channels (FIG. 3).

More than 70 genes for K^+ channel α subunits have been identified in mammals. Their common feature is a

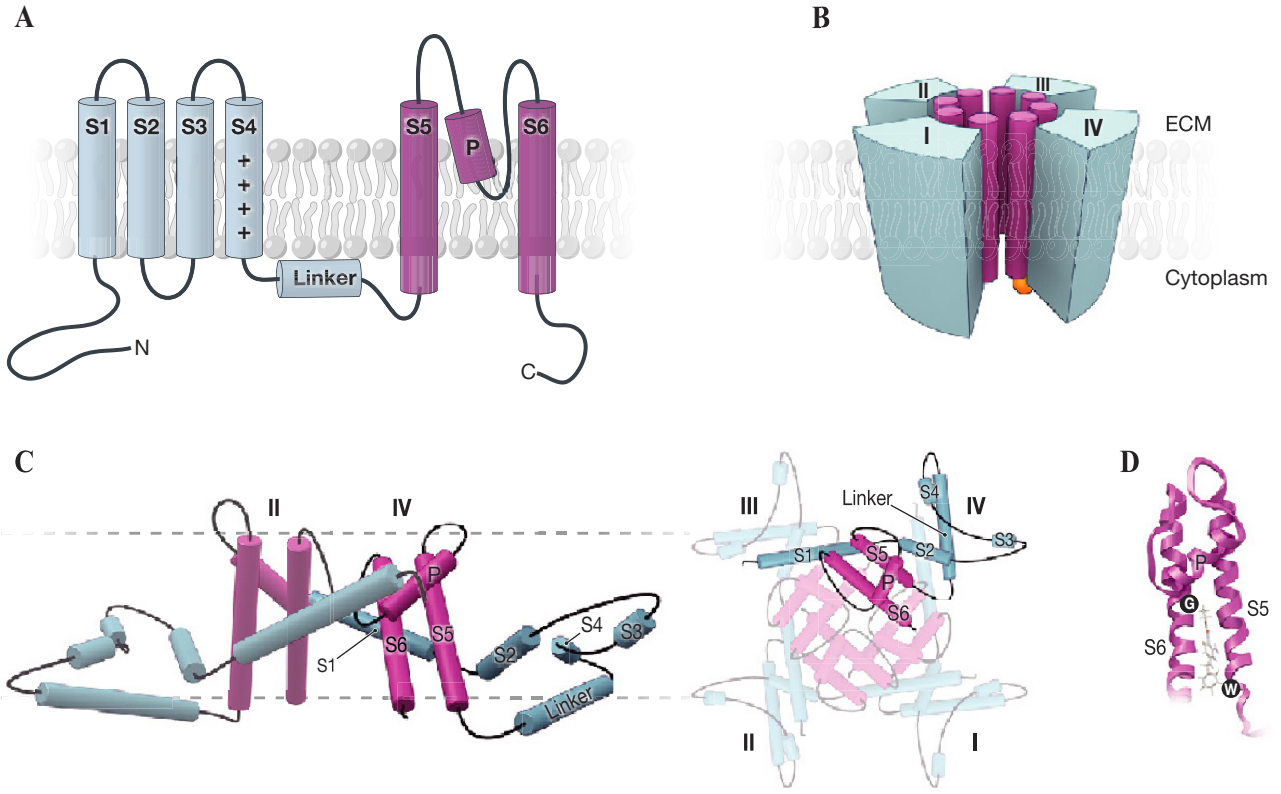

FIG. 3. Voltage-gated K^+ channels. **A:** Membrane topology of the α subunit of voltage-gated K^+ channels. The voltage sensor domain in S4, containing four positively charged arginine residues (blue-gray), is connected to the pore-domain (red) by the gating linker. **B:** Perspective diagram showing how the S5–S6 transmembrane elements form, with their P-loops, a ring surrounding the ion-selective pore. **C:** Diagrams showing the spatial disposition of the helical elements of the α subunits of the voltage-gated K^+ channel, as determined by X-ray crystallography.[429,430] *Left:* Lateral view of the membrane showing disposition of helical elements of two α subunits. *Right:* External view of the pore-forming domains of four α subunits and their associated voltage-sensing domains. **D:** Ribbon diagram of the S5, S6, and P-loop helices, showing the docking site for retigabine (molecule shown in gray beside the tryptophan residue W236 on S5). G indicates the glycine G301 that provides the gating hinge in S6. Part C is adapted from refs. Long et al.,[429,430] with permission. Part D is adapted from Wuttke et al.,[152] with permission.

sequence motif TVGYG in the P-loop that confers selectivity of the pore for K^+ over Na^+. K^+ channels fall into several families: the six-transmembrane-helix voltage-gated (K_v) channels, the two-transmembrane-helix inward-rectifier (K_{ir}) channels, the Ca^{2+}-activated K^+ channels (K_{Ca}), and the tandem-pore domain (K_{2P}) channels. Tandem-pore domain K^+ channels are comprised of two subunits each containing two P-loops, thus forming a channel homogenous to the tetrameric structure of other K^+ channels.

The K_v and K_{Ca} families are of particular relevance in epilepsy. The K_v family has more than 40 members,[108] and the channels that they form include the delayed-rectifier K^+ current (I_K) responsible for the repolarization phase of action potentials, as well as other voltage-gated K^+ currents that have diverse functions in neurons, including the A-current (K_v1, K_v3, and K_v4) and the M-current (KCNQ).

A-currents and M-currents play important roles in regulating the excitability of neurons in brain regions relevant to epilepsy, such as the neocortex and the hippocampus.[109] K_v channels are formed as homomeric or heteromeric assemblies within groups K_v1 (delayed-rectifier and A-current), K_v2 (delayed rectifier), K_v3 (high-voltage-activated, fast kinetics), K_v4 (somatodendritic A-current), and K_v7 (M-current), as well as ether-à-go-go channels, including the eag, elk (eag-like), and erg (eag-related) subrelated. K_v2 channels can combine with "silent" nonexpressing modifier α subunits designated as $K_v5.1$, $K_v6.1$, $K_v8.1$, and $K_v9.1$–$K_v9.3$.

The calcium-activated K^+ channels fall into two subfamilies,[110] one of which includes three small-conductance K_{Ca} channels ($K_{Ca}2.1$–$K_{Ca}2.3$; also designated SK1–SK3). The other includes a large-conductance channel ($K_{Ca}1.1$; also known as the BK or Slo1 channel), which is anomalous in having seven transmembrane segments and an extracellular N-terminus.

Two further K^+ channel families are functionally and structurally distinct. The simplest are the inwardly rectifying K_{ir} channels,[111] which have a pore-forming P-loop between two transmembrane segments (M1 and M2), analogous to the P-loop between the S5 and S6 segments in six transmembrane domain channels. The fourth K^+ channel family that underlies much of the

"leak" currents in neurons is referred to as the two-pore family (K_{2P}) or the "four-transmembrane-family," because the gene products encode two of the basic pore-forming units that combine as a dimer to produce the functional channels (known as the TWIK, TASK, TRAAK, and TREK channels).[112] In all four families, four P-loops come together to form the outer mouth of the ion channel pore.

The auxiliary subunits vary markedly between the K^+ channel families.[113] Thus, the voltage-gated K^+ channels have intracellular β subunits (β1–β3) that bind to the α subunits in a symmetrical fashion forming an octameric channel. The N-terminal of the β subunits acts as an inactivation gate for the K_v1 α subunits. Four further intracellular proteins (K_v channel-interacting proteins, KChIP1-4) that are calmodulin-like regulatory proteins interact with K_v4 channels. A further auxiliary subunit KCNE (mink-like) has a single transmembrane helical segment and regulates function in KCNQ channels (K_v7). K_{Ca} channels are associated with $K_{Ca}\beta$ subunits ($K_{Ca}\beta$1–4) that have two transmembrane segments. One subfamily of K_{ir} channels, the K_{ATP} or K_{ir}6.x channels, is regulated by associated sulfonylurea receptors (SUR1, SUR2A/B) that have two ATP binding motifs.

TABLE 3 summarizes the K^+ channel genes that to date have been associated with epilepsy. The first such gene was *Kcna1* (K_v1.1), which, when disrupted in mice, results in a phenotype reminiscent of limbic epilepsy beginning at 3 weeks of age.[114] CA3 pyramidal neurons in brain slices taken from these animals exhibit hyperexcitability. Episodic ataxia type 1 (EA1)—an autosomal dominant disorder involving both the central and the peripheral nervous system characterized by attacks of ataxia and persistent myokymia—is associated with point mutations in the K_v1.1 (*KCNA1*) ion channel. There is a high incidence of epilepsy, including complex partial seizures, among individuals with this syndrome, indicating that *KCNA1* is an epilepsy susceptibility locus.[115]

A second example of a mutation in a K_v subunit associated with epilepsy is in a developmental syndrome associated with a deletion on chromosome segment 1p36 that includes the auxiliary β2 subunit *KCNAB2*. Many of the individuals with this syndrome exhibit partial or generalized seizures and infantile spasms.[116] Inasmuch as mice in which the β2 subunit has been deleted by gene targeting exhibit seizures, loss of the β2 subunit is likely to be the cause of seizures in the human developmental syndrome.

The way in which deletion of the β2 subunit may alter K^+ currents has not yet been defined. A presumed tumor suppressor gene *LGI1* (leucine-rich glioma inactivated 1) has been found to be associated with autosomal dominant lateral temporal lobe epilepsy with auditory features. It has now been shown that the LGI1 protein coassembles with K_v1.1, K_v1.4, and $K_v\beta$1 subunits in axon terminals in the hippocampus.[35] LGI1 normally prevents inactivation of the channel by the β subunit, but when it is defective, inactivation of the A-type currents is abnormally rapid. This provides a further example of a reduction in a voltage-dependent K^+ current resulting in focal (limbic) epilepsy.

The first K^+ channel gene to be definitively linked to a human epilepsy syndrome was *KCNQ2* (and shortly thereafter *KCNQ3*).[117] Mutations in these genes were found in the autosomal dominant syndrome benign familial neonatal convulsions (BFNC).[118,119] In addition, mice in which a single copy of the gene for KCNQ2 was disrupted by gene targeting (*Kcnq2*$^{+/-}$) showed increased sensitivity to PTZ seizures.[120] The combination of KCNQ2 and KCNQ3 underlies the bulk of the M-current in neurons,[121] although KCNQ5 alone or in combination with KCNQ3 can also contribute to M-current. It is believed that M-current regulates neuronal excitability by determining the neuronal firing threshold, influencing the firing rate, and modulating neuronal responsiveness to synaptic inputs. KCNQ2 (K_v7.2) and KCNQ3 (K_v7.3) can form homomeric or heteromeric channels, which are expressed on the axons or soma of neurons. KCNQ2 is also responsible for a slow K^+ current that regulates excitability in neurons and axons.[122]

Studies with expressed mutated channel subunits show that BFNC mutations result in a loss of K^+ currents, making BFNC a haploinsufficiency syndrome. Although the neonatal seizures in BFNC resolve by 3 months of age, BFNC is associated with an enhanced incidence (up to 16%) of various forms of epilepsy later in life, so that *KCNQ2* and *KCNQ3* can be considered epilepsy susceptibility genes.

Pharmacological blockade of large-conductance Ca^{2+}-activated K^+ channels (K_{Ca}1.1 or BK) does not lead to seizures, unlike blockers of K_v channels.[22] Moreover, mutations that reduce the function of K_{Ca} channels have not been associated with epilepsy syndromes. BK channels contribute to the fast after-hyperpolarization in neurons, and the observation that inhibiting their activity does not lead to seizures indicates that they have a functional role distinct from that of K_v channels. It was a surprise, therefore, when mutations in the *KCNMA1* gene, which encodes the α subunit of K_{Ca}1.1, were associated with a syndrome of generalized epilepsy and paroxysmal dyskinesia.[123]

In contrast to typical K^+ channelopathies associated with epilepsy, in which there is a loss of function, mutant BK channels exhibited a gain of function. Indeed, the channels conducted markedly greater macroscopic current due to an increase in the single-channel open probability and an enhancement in their Ca^{2+} sensitivity. (BK channels are activated by depolarization and by Ca^{2+}.) It was hypothesized that the enhanced BK channel activity

causes a more rapid repolarization of action potentials, allowing neurons to fire at faster rates and thus enhancing seizure susceptibility. In contrast to BK channels, which mediate fast spike repolarization, small conductance (SK) K_{Ca} channels (K_{Ca}2.1, 2.2, and 2.3) do not play a role in spike repolarization, but rather generate slow after-hyperpolarizations.

Blockade of SK channels with apamin can lead to epileptiform activity, at least in *in vitro* hippocampal slice preparations.[124] In contrast to Kv blockers, however, intracerebroventricular injection of apamin does not lead to frank seizure activity. Conversely, enhancing SK channel activity with 1-ethyl-2-benzimidazolinone (EBIO), which activates all three SK channels, inhibits epileptiform activity in the hippocampal slice.[125] Consequently, both BK and SK could represent potential targets for AED drugs. However, agents that affect BK channels are unlikely to be of widespread utility in epilepsy, although BK blockers that have similar selectivity to paxilline[22] might be specifically useful in generalized epilepsy with paroxysmal dyskinesia (GEPD). SK activators might have wider utility, but optimism is guarded, given that apamin is not a particularly powerful convulsant.

Various genetic studies have linked inwardly rectifying K^+ channels with generalized seizures in rodents and in humans. For example, an association study linked a polymorphism in *KCNJ3* with absence seizures.[126] *KCNJ3* encodes K_{ir}3.1, a channel that induces membrane hyperpolarization in response to activation of G-protein linked receptors.[126] *Weaver* mice show severe ataxia with loss of cerebellar granule cells and sometimes generalized convulsions. They have a mutation in *Kcnj6* causing the pore-forming domain of the G-protein activated channel Girk2 to lose ion selectivity.[127] *Kcnj6* knockout mice show reduced expression of Girk1 and Girk2 and spontaneous seizures, but not impaired cerebellar development.[128]

Polymorphisms relating to another inwardly rectifying K^+ channel, K_{ir}4.1 (*KCNJ10*), are proposed as conferring seizure sensitivity or resistance in inbred mouse strains on the basis of differences observed in *Kcnj10* in DBA/2 *versus* C57BL/6 mice and confirmed in other strains showing varying seizure sensitivity.[129] Polymorphisms in the same *KCNJ10* have also been found in humans, where they differentiate between patients with epilepsy and controls.[130] Finally, a mutation in the K_{2P} channel gene *KCNK9* has been found in the rat model of human absence epilepsy referred to as GAERS (genetic absence epilepsy rats from Strasbourg). *KCNK9* encodes the TASK3 (TWIK-like acid-sensitive K^+) channel.[131] No functional correlate of the mutation has been identified.

Classical pharmacological antagonists of K_v channels include 4-aminopyridine (4-AP), which is commonly used to induce seizures in rodent models[23] and brain slices[132] and is a blocker of K_v1, K_v3, and K_v4 channels. A variety of peptide toxins from snakes, sea anemones, and scorpions also selectively block K_v channels. These toxins bind with high affinity near the pore region of the K_v channel, blocking current flow.[133,134] The best known of these toxins are the dendrotoxins found in the *Dendroaspis* genus of the African mamba snake, which induce behavioral and electrographic seizure activity when injected intracerebrally and are also active in brain slice preparations.[135,136] The dendrotoxins block K_v1.1 and also K_v1.2 and K_v1.6. The scorpion toxin tityustoxin-Kα also blocks K_v channels by binding at the same site as dendrotoxin[137] and similarly induces seizures.[22] Pandinustoxin-Kα seems to preferentially inhibit A-type currents and also induces seizures when injected intraventricularly.[22]

There are several classes of compounds that have been identified as K^+ channel openers and which could potentially have anticonvulsant activity. The first class to be described were K_{ATP} (K_{ir}6.x) channel openers, such as cromakalim and diazoxide. These agents did not prove to have activity in conventional AED screening models (M.A. Rogawski, unpublished observations).[138] There is, however, one report that cromakalim can inhibit epileptic discharges in brain slices,[139] and two additional brief reports that it can inhibit seizures induced by a K^+ channel toxin[140] and reduce the frequency of spike-and-wave discharges in WAG/rij rats when injected intracerebroventricularly.[141]

Because K_{ATP} channels may be activated mainly under conditions of neuronal energy failure (they are inhibited by ATP), such as in anoxia, openers of K_{ATP} channels are unlikely to have broad utility in epilepsy therapy, although they might in theory be useful for the treatment of anoxia-induced seizures.[142,143] K_{ATP} channels are, however, present in peripheral tissues, including the heart and vasculature; to date, no K_{ATP} opener has been found that does not have adverse cardiovascular effects due to actions on these peripheral channels.

The second class of K^+ channel opener to be described is one that acts selectively on neuronal KCNQ (M-current) channels. Retigabine was the first compound to be identified with this activity, but flupirtine, the analog upon which the synthesis of retigabine was based, is now known to also be an opener of KCNQ channels.[144] Retigabine, originally believed to be a GABA modulator, was shown to be anticonvulsant in a wide range of animal models of epilepsy.[145] In fact, it does potentiate inhibitory postsynaptic currents (IPSCs) through an action on $GABA_A$ receptors[146]; however, the extent to which an action on GABA-mediated inhibition contributes to the anticonvulsant activity of retigabine has not been defined. In 1997, Rundfeldt[147] reported that retigabine activates a K^+ current in slightly depolar-

ized NG108-15 cells, and, in 2000, three research groups independently demonstrated that the current affected was the M-current carried by $K_v7.2$ or $K_v7.3$ (whose channels are encoded by *KCNQ2* and *KCNQ3*, respectively).[148–150]

Retigabine causes a large hyperpolarizing shift in the voltage-dependence of activation of these channels. As a result, there is greater K^+ current at the resting membrane potential, which stabilizes the resting potential toward the K^+ equilibrium potential (E_K), reducing neuronal excitability. Retigabine was subsequently shown to activate all four KCNQ isoforms expressed in the brain (KCNQ2–5), but not the isoform responsible for the cardiac M-current (KCNQ1).[151] Study of chimeras derived from KCNQ1–KCNQ2[152] or KCNQ1–KCNQ3[151] showed that S5, S6, and the pore loop contribute to retigabine sensitivity. Point mutations further indicated that a tryptophan residue on the cytoplasmic end of S5 is essential for the action of retigabine. It was proposed that retigabine binds to a hydrophobic pocket formed when the channel opens and that this explains the strong shift in the voltage-dependence of activation.[152,153] Several other classes of compounds have been shown to act as KCNQ2–5 channel openers, including benzamides, benzisoxazoles, and phenylacrylamides.[25,138] Some of these protect against seizures in animal models and are more specific for KCNQ channels than is retigabine, confirming that opening of KCNQ channels per se is an anticonvulsant mechanism.

Actions of several established and novel AEDs on various K^+ currents have been reported, but it is difficult to assess their significance. For example, ethosuximide was claimed to reduce a sustained K^+ current in thalamic neurons, an effect interpreted as a block of a Ca^{2+}-activated K^+ current.[154] In addition, pregabalin was reported to open ATP-sensitive K^+ channels.[155] Lamotrigine, however, was found to reduce the amplitude of A-type K^+ currents in cultured hippocampal neurons[156] and levetiracetam was reported to inhibit delayed rectifier but not A-type K^+ currents in isolated hippocampal neurons.[157] These inhibitory actions would be expected to enhance excitability and are unlikely to contribute to anticonvulsant activity. Lamotrigine also blocks hERG, the human ether-à-go-go K^+ channel (KCNHZ), that would be proarrhythmic in the heart[158] and might favor sudden unexpected death in epilepsy (SUDEP), which is a significant problem in adults with poorly controlled seizures. SUDEP may be related to cardiac arrhythmias and, although it is more frequent in patients on AED polytherapy,[159] there is no direct evidence linking SUDEP to AEDs.

In summary, there is clear evidence that voltage-gated K^+ channels are valid molecular targets for AEDs. Indeed, it is likely that retigabine or another drug activating $K_v7.2$–7.5 channels will be introduced into clinical practice. The K_v1, K_v3, and K_v4 channels that underlie A-type currents are also attractive AED targets, as are inwardly rectifying K^+ channels. Although AEDs acting on K^+ channels might have utility in the treatment of many seizure types, it is tempting to speculate that they might be especially effective in epilepsy syndromes associated with mutations affecting K^+ channels.

There are no studies examining the activity of retigabine in treating seizures associated with genetic defects that cause reduced function of retigabine-sensitive KCNQ channels; however, a mouse mutant *Szt1* has been described in which there is deletion of the genomic DNA encoding the *KCNQ2* C-terminus, along with other genes. Heterozygotes at this locus have reduced M-current in hippocampal CA1 neurons and the residual M-current is markedly less retigabine sensitive.[160] Although *Szt1* mice have reduced seizure thresholds,[161] they also have diminished sensitivity to retigabine, as expected from the studies of M-current in these animals. This finding serves as a reminder of the fact that genetic alterations in AED targets can alter drug sensitivity, and in some instances the impact may be to cause pharmacoresistance.

HCN channels

The hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels are Na^+-permeable and K^+-permeable channels that participate in pacemaker currents in cardiac cells and neurons. The channels are opened by hyperpolarization to negative membrane potentials (more negative than –50 mV). In addition, they are also modulated by cAMP binding to a consensus cyclic nucleotide binding domain in the carboxy terminus. Binding of cAMP shifts the voltage dependence of activation to more positive potentials; it can also directly open the channels.

There are four known subunits, HCN1–4, each of which has six transmembrane segments. The subunits combine to form homomeric or heteromeric tetramers, as do K_v channels. HCN1 channels are prominently expressed in the cortex and the hippocampus, particularly in dendrites. In contrast, HCN2 channels, which are highly responsive to cAMP, are expressed mainly in the thalamus, where they are believed to limit burst firing. In recordings from hippocampal or cortical neurons, the current produced by opening of the HCN channels is referred to as I_h. Many neurotransmitters (including monoamines, serotonin, and acetylcholine) can modulate I_h through cAMP.

A role for HCN channels in epilepsy has been widely proposed, but the evidence is complex.[162] No spontaneous mutations in HCN channels have been identified in epilepsy. In mice, deletion of the HCN2 subunit produces animals with 5-Hz spike-and-wave discharges and absence-like seizures.[163] The WAG/Rij rat model of ab-

sence epilepsy shows a loss of HCN1 function in the cortex[164] (possibly linked to the origin of cortical spike-and-wave discharges) but enhanced HCN1 expression in the thalamus.[165] In addition, changes in I_h and in HCN subunit expression have been observed in epileptogenesis. Following febrile seizures in infant rodents, there is a prolonged increase in I_h,[166,167] but after kainate seizures there is a reduction in I_h in entorhinal cortex layer III neurons.[168]

I_h is an attractive potential AED target for different types of epilepsy. ZD-7288, a blocker of HCN channels, inhibits spontaneous epileptiform bursting in the hippocampal slice, confirming the potential of I_h inhibition as an anticonvulsant approach.[169] Because the HCN isoforms have distinctive regional expression patterns and functions, the subunit selectivity of a potential drug may be of significance. Drugs targeting HCN1 might be relevant for limbic seizures, whereas those affecting HCN2 may be more relevant to absence epilepsy. In rat hippocampal pyramidal neurons, lamotrigine has been reported to decrease dendritic excitability by increasing I_h.[170] If similar effects occur on HCN channels in thalamocortical networks, this activity could potentially account for the efficacy of lamotrigine in absence epilepsy.

Voltage-gated chloride channels

The mammalian ClC gene family encodes nine Cl^- channels with diverse functions in plasma membranes and intracellular organelles. One of these channels, ClC-2, a homodimeric channel found in neurons and glia (encoded by the *CLCN2* gene), has been implicated in epilepsy.[171] ClC-2 is a plasma membrane channel activated by hyperpolarization, cell swelling and extracellular acidification. ClC-2 knockout mice do not have epilepsy.[172] In humans, however, ClC-2 mutations cosegregated in three families with various idiopathic generalized epilepsy syndromes, including JME, juvenile absence epilepsy, CAE, and epilepsy with grand mal seizures on awakening (EGMA).[173] Functional studies in transfected cells suggest that the mutations cause a loss of function.[174] At present, there is no definitive evidence that these mutations are responsible for the epilepsy syndromes; searches for ClC-2 mutations in other cohorts with epilepsy revealed sequence changes that were probably only polymorphisms. In any case, it has been proposed that the epilepsy-associated ClC-2 mutations may lead to alterations in the Cl^- gradient in neurons such that $GABA_A$-mediated inhibition is impaired or may even become excitatory.

Even though ClC-2 is not a likely AED target, anticonvulsant strategies that attempt to influence Cl^- gradients by altering the activity of the transporters that determine Cl^- gradients (NKCC1 and KCC2) comprise an active area of investigation, given the widespread expression of ClC-2 in many tissues. The importance of these transporters in the regulation of seizure susceptibility is highlighted by the seizure phenotypes of mice deficient in KCC2, a neuronal electroneutral K^+ and Cl^- cotransporter that drives intracellular Cl^- to low concentrations and shifts the reversal potential for $GABA_A$ and glycine receptors so that the channels are hyperpolarizing. Mice lacking KCC2 exhibit severe seizures and die shortly after birth,[175] whereas those in which KCC2 has been reduced by 80–85% show enhanced susceptibility to PTZ seizures.[176]

LIGAND-GATED ION CHANNELS

Ligand-gated ion channels in the mammalian brain fall into two major superfamilies, the cys-loop receptors (comprising the $GABA_A$, glycine, nicotinic, cholinergic, and 5-HT3 receptors) and the glutamate ionotropic receptors (comprising the AMPA, kainate, and NMDA receptors). There are two additional superfamilies: the ATP-gated P2X channels and the TRP channels. Binding of agonist to these receptors induces a conformational change that opens the channel.

The cys-loop receptors vary in their ion selectivity. $GABA_A$ and glycine receptors are permeable to Cl^- and HCO_3^-, whereas nicotinic cholinergic receptors are permeable mainly to Na^+ and K^+, but also to Ca^{2+}. The ionotropic glutamate receptors are also cation permeable, with significant variation in the extent of Ca^{2+} permeability. The majority of known convulsant compounds (natural or synthetic) act via the ligand-gated ion channels, with the greatest number and variety acting to diminish GABA-mediated transmission either by direct action on $GABA_A$ receptors or by other effects on GABAergic function.

Cys-loop ligand-gated channels

$GABA_A$ receptors. GABA serves as the main fast inhibitory neurotransmitter in the brain. Inhibitory interneurons that make use of GABA as their neurotransmitter are found throughout the brain, but in any region they may comprise a wide range of morphological and functional types that participate in different circuits with principal neurons. Thus, in the CA1 area of the rat hippocampus it is possible to distinguish 16 different types of GABAergic interneurons on the basis of their morphology, specific protein content (e.g., calbindin, calretinin, parvalbumin), and pattern of firing in relation to ongoing rhythms and oscillatory firing of pyramidal neurons.[177] Through the mechanism of recurrent inhibitory feedback, GABAergic interneurons in the cortex terminate local sustained burst firing and, through inhibitory surround, limit the lateral spread of seizure activity. Chemical agents that impair GABAergic inhibition are powerful convulsants.

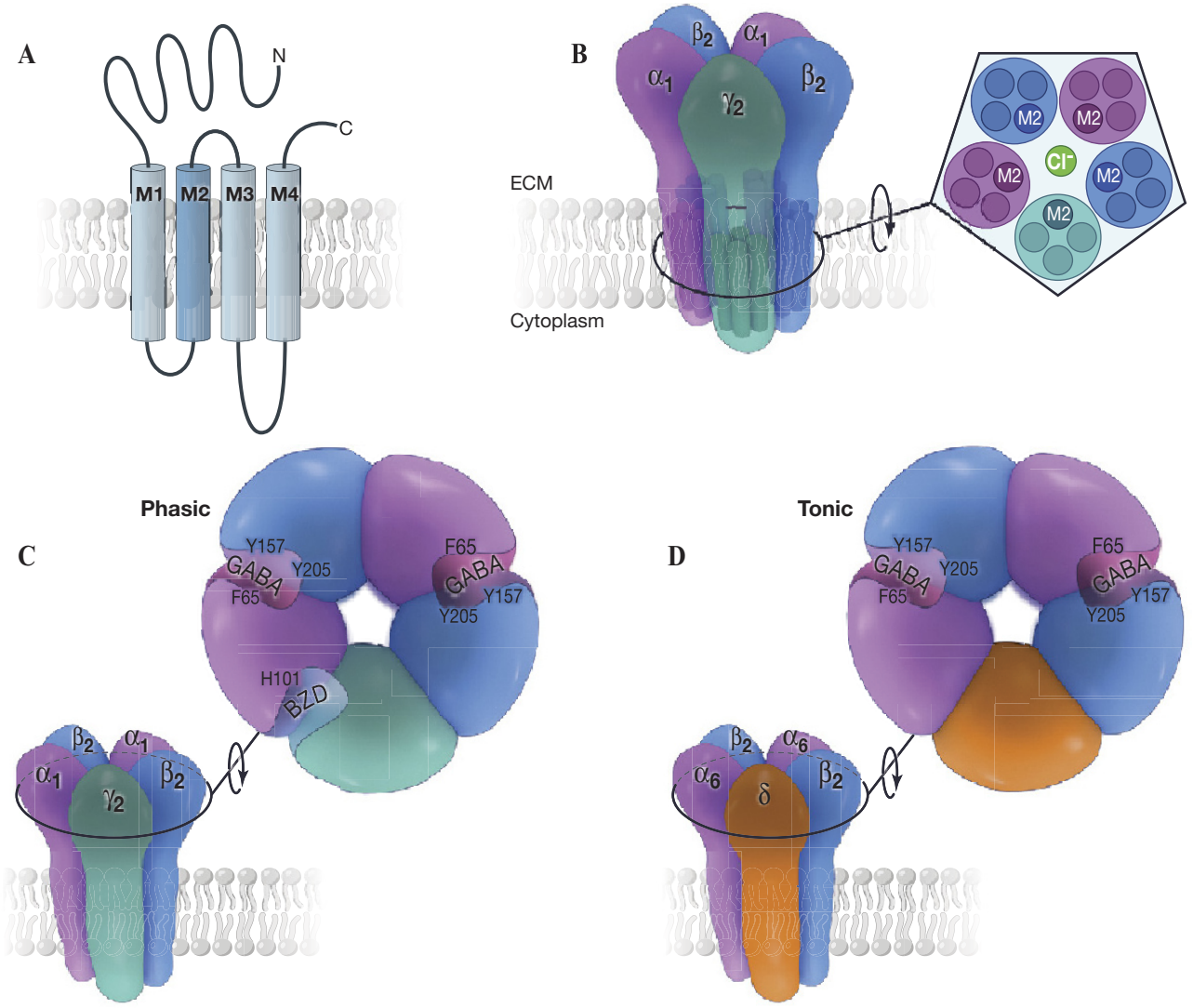

FIG. 4. The $GABA_A$ receptor. **A:** Membrane topology diagram of a $GABA_A$ receptor subunit showing four transmembrane segments and extracellular NH_2 and COOH termini. **B:** Characteristic pentameric $GABA_A$ receptor structure composed of two α, two β, and one γ subunits. A cross-section of the intramembrane structure shows the Cl^- channel pore formed by M2 helical elements. **C:** A typical synaptic (phasic) $GABA_A$ receptor with a view of the extracellular face showing the two recognition sites for GABA (at the junctions between α and β subunits) and the benzodiazepine recognition site (at the junction between an α and the γ subunit). **D:** A typical extrasynaptic (tonic) $GABA_A$ receptor with a δ subunit replacing the γ subunit in the phasic channel. The extracellular face shows the two GABA recognition sites.

$GABA_A$ receptors are pentameric in structure, with the five subunits arranged like spokes of a wheel around a central Cl^--selective pore[178] (FIG. 4). The 19 subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, ρ1–2) are encoded by 19 distinct genes. Each subunit has four transmembrane segments, with both the amino and carboxy termini located extracellularly. These extracellular segments form the recognition sites (two per channel) for GABA and also, in some channel types, the recognition site (one per channel) for benzodiazepine-like allosteric modulators. The subunit composition determines both the biophysical properties of the receptor–channel complex and its pharmacology, most notably the sensitivity to benzodiazepines.[179,180] A typical benzodiazepine-sensitive $GABA_A$ receptor consists of two α1, α2, α3, or α5 subunits, two β2 or β3 subunits (or one each), and a γ2 subunit (FIG. 4).

Classically, $GABA_A$ receptors have been recognized as mediating phasic (synaptic) inhibition through the generation of fast, transient, rapidly desensitizing currents (IPSCs) in postsynaptic neurons in response to synaptically released GABA. More recently, it has been recognized that $GABA_A$ receptors also contribute to tonic (extrasynaptic) inhibition, representing the Cl^- conductance activated at nonsynaptic sites in response to background concentrations of GABA.[181]

Phasic and tonic inhibition are mediated by $GABA_A$ receptors with different subunit composition, GABA af-

TABLE 4. *Subunits of the $GABA_A$ Receptor: Gene Defects in Epilepsy and Pharmacology*

Human Gene	Subunit	Epilepsy Syndrome		Benzodiazepine Action	Positive Allosteric Modulators/Agonists
		Mouse	Human Mutation		
GABRA1, *GABRA2*, *GABRA3*, *GABRA5*	α1, α2, α3, α5		JME (α1 A322D)	Sensitive	Barbiturates, benzodiazepines, felbamate, topiramate
GABRA4, *GABRA6*	α4, α6			Insensitive	Neurosteroids, gaboxodol (THIP)
GABRB1, *GABRB2*, *GABRB3*	β1, β2, β3	β3 knockout model of Angelman syndrome			Etomidate, propofol
GABRG2	γ2		GEFS+ (K289M, Q351X); CAE+FC (R34Q, IVS6+2T-G)	Sensitive	
GABRD	δ		GEFS+ (E177A, R220C)		Neurosteroids

Benzodiazepines act as positive allosteric modulators of sensitive isoforms. High concentrations of barbiturates, propofol, etomidate, neurosteroids and topiramate directly activate $GABA_A$ receptors, whereas benzodiazepines and felbamate do not.
CAE+FC = childhood absence epilepsy and febrile convulsions; GEFS+ = Generalized epilepsy with febrile seizures plus (type 3); JME = juvenile myoclonic epilepsy.

finities, and rates of desensitization. The most notable difference in subunit composition is that the receptors mediating tonic inhibition contain the δ subunit, rather than the γ subunit characteristic of synaptic $GABA_A$ receptors.[182] Receptors containing α4, α5, or α6 are commonly found nonsynaptically. Pharmacologically, the most notable difference is that receptors with α4, α6, or δ subunits are not potentiated by benzodiazepines or by nonbenzodiazepine benzodiazepine receptor agonists (such as zolpidem), whereas those with α1, α2, α3, α5, or γ2 subunits are benzodiazepine sensitive (TABLE 4). The benzodiazepine-sensitive α subunits (α1, α2, α3, α5) differ from the insensitive ones (α4, α6) in possessing a histidine residue at position 101.

Genetic studies in humans reveal a range of idiopathic generalized epilepsy syndromes linked to mutations in the $GABA_A$ receptor[183] (TABLE 4). We note that these syndromes may be phenotypically indistinguishable from those associated with mutations in voltage-gated ion channels. For example, a mutation in the $GABA_A$ receptor α1 subunit is associated with autosomal dominant juvenile myoclonic epilepsy[184]; the mutation reduces peak current by decreasing trafficking of the subunit, so that there is deficient surface expression.[183] Mutations involving the γ2 subunit in two cases associated with GEFS+ and in two cases associated with childhood absence epilepsy with febrile convulsions have been found to alter the kinetic properties of the receptor and also their surface expression.[183] Three mutations in families with GEFS+ involving the δ subunit[185] are associated with reduced current, suggesting that impairment of tonic inhibition by extrasynaptic GABA receptors can also produce epilepsy. Studies in genetically modified mice have revealed spontaneous seizures in β3 knockout mice,[186] supporting the interpretation that the seizures that are a prominent feature of the Angelman syndrome (which, in addition to other genetic abnormalities, lacks the β3 gene) are due specifically to defects in $GABA_A$ receptors.

Studies in genetically modified mice have also helped establish the role played by subunit composition in the antiepileptic and other pharmacological actions of drugs acting on the $GABA_A$ receptor.[187] These studies indicate that seizure protection conferred by benzodiazepine-like agents depends primarily on $GABA_A$ receptors composed of α1 subunits, and also those containing α2, α3, or α5 subunits. In contrast, sedative actions are mediated primarily by receptors containing α1 subunits, anxiolytic actions primarily by receptors containing α2 subunits, and myorelaxant actions by receptors with α2, α3, and α5 subunits. Therefore, drugs that selectively target $GABA_A$ receptors containing α2 or α3 can be expected to avoid sedative side effects.

In practice, although such agents do appear to exhibit anticonvulsant activity in animal models, human trials to date have not shown that selective targeting can avoid the troubling sedative activity of nonselective benzodiazepine agonists.[10] Mice lacking δ subunits exhibit spontaneous seizures and greater sensitivity to the convulsant PTZ, demonstrating a role of the δ subunit containing $GABA_A$ receptors in regulating seizure susceptibility.[188] Such $GABA_A$ receptors are particularly sensitive to modulation by neurosteroids, and this effect of neurosteroids may be lost in δ knockout animals.[189,190]

TABLE 5. *Functional Actions of Positive $GABA_A$ Receptor Modulators*

Compound Type	Direct Action	GABA Potentiation	GABA Current Type Affected	Actions on Glutamate Receptors
Benzodiazepines (diazepam, clonazepam, lorazepam)		++	Phasic	
Gaboxadol	++		Tonic	
Barbiturates (phenobarbital)	+	++	Phasic/tonic	AMPA
Chlormethiazole	+	++		
Neurosteroids (ganaxolone)	+	++	Tonic	
Etomidate		++		
Propofol		++		
Loreclezole	+	++		
Ethanol		+	Tonic	
Topiramate		+		Kainate (GluR5)/AMPA
Felbamate		+		NMDA

"Direct action" indicates gating of $GABA_A$ receptor in the absence of GABA. "GABA potentiation" indicates modulation of $GABA_A$ receptor responses in the presence of GABA. Tonic GABA currents are mediated by extrasynaptic $GABA_A$ receptors; phasic currents are synaptic. Last column indicates additional known actions on ionotropic glutamate receptors.
+ = modest effect; ++ = robust effect.

An extraordinary variety of chemical compounds are capable of acting on $GABA_A$ receptors to directly influence their Cl^- conductance or modulate gating by GABA (TABLE 5). Those that mimic or potentiate the effects of GABA are generally sedative or anesthetic, and have anticonvulsant actions. Those that block the action of GABA or allosterically inhibit receptor function (as with benzodiazepine site inverse agonists such as DMCM [methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate]) are proconvulsant. GABA analogs, such as muscimol and gaboxadol (THIP [4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol]), act as GABA recognition site agonists. They are not useful systemically as anticonvulsants, inasmuch as muscimol is psychotomimetic and gaboxadol (which has a greater potency on receptors containing the δ subunit[191]) is sedative. Both muscimol and gaboxadol can have both anticonvulsant and proconvulsant actions when injected into the brain, the specific effect depending on the site of injection and the developmental age.[192–196]

The majority of drugs that act on $GABA_A$ receptors do so at modulatory sites distinct from the GABA recognition site.[179] Classical benzodiazepines are used to treat status epilepticus, but have limited utility in the chronic treatment of epilepsy because of the development of tolerance. Nonbenzodiazepine compounds, such as zolpidem, that are selective for α1 (and also α5 subunits) are sedative and are not suitable for epilepsy therapy. Similarly, the β2/β3 selective agent loreclezole has sedative properties.[197,198]

Substantial effort has been devoted to obtaining $GABA_A$ receptor positive allosteric modulators that have reduced activity on GABA receptors containing α1 subunits, to avoid the sedation that is believed to be mediated by these receptors. The compounds developed so far are generally nonbenzodiazepines that bind to the benzodiazepine site on all benzodiazepine-sensitive isoforms, but at certain isoforms are partial agonists with reduced efficacy. For example, TPA023 [7-(1,1-dimethylethyl)-6-(2-ethyl-2*H*-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine], which selectively modulates α2- or α3-containing receptors, is anxiolytic yet not sedating in rodents.[199] ELB139 [1-(4-chlorophenyl)-4-piperidin-1-yl-1,5-dihydro-imidazol-2-on], which is selective for α3-containing receptors, also has anxiolytic and anticonvulsant properties but is nonsedating in rodents.[200] Abecarnil (isopropyl-6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate), a full agonist at α3-containing receptors and a partial agonist at α5-containing receptors, has a markedly different spectrum of activity from diazepam in animal models of epilepsy, being less active against MES and bicuculline seizures in mice but more active against DMCM seizures in mice and photically induced seizures in *Papio papio*.[201] It is also much less myorelaxant.

These various subtype-selective agents could potentially be superior to benzodiazepines for chronic epilepsy therapy, but it remains to be demonstrated that they are less sedative in humans than classical benzodiazepines and, importantly, that they are less susceptible to tolerance. There is some cause for optimism regarding the issue of tolerance, which is reduced for drugs that act as partial agonists.[202,203] At the same time, however, there is also evidence that rodent models do not adequately predict liability for sedation, so that agents demonstrated to be nonsedative in rodents may cause sedation in humans.[25]

The emerging understanding of the different roles of phasic and tonic inhibition in epileptic phenomena may suggest improved approaches to targeting $GABA_A$ receptors for epilepsy therapy.[204] For example, neuroactive steroids selectively target $GABA_A$ receptors containing δ

subunits that mediate tonic inhibition[205,206] (TABLE 5). They may have reduced liability to tolerance[207] and may be useful in specific epilepsy syndromes, such as infantile spasms, or to treat hormone-dependent seizure exacerbations, such as in catamenial epilepsy.[208,209]

Nicotinic cholinergic receptors. Like other members of the cys-loop receptors, nicotinic cholinergic receptors are pentameric structures. In mammals, the 16 subunits (α1–7, α9, α10, β1–β4, δ, ε, and γ), each of which has four transmembrane domains, are encoded by 16 distinct genes. Neuromuscular junction receptors are composed of α1 subunits; brain neuronal receptors are composed of α4 or α7 subunits. The brain receptors act presynaptically to modulate transmitter release by increasing Na^+ and Ca^{2+} entry,[210] and they are thought to play an important role in cognitive functions.

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), characterized by partial seizures that occur predominantly during sleep,[211] has been associated in some families with mutations involving either the α or β subunit in $\alpha4\beta2$ nicotinic acetylcholine receptors.[212–214] A common feature of the ADNFLE mutations is that they reduce Ca^{2+} potentiation of the $\alpha4\beta2$ nicotinic receptor response to acetylcholine. It has been proposed that the decrease in Ca^{2+} potentiation could contribute to ADNFLE seizures by reducing presynaptic nicotinic receptor activation of inhibitory transmitter release in the cortex or by shifting the balance between nicotinic receptor-induced excitatory and inhibitory transmitter release during bouts of high-frequency cortical synaptic activity in favor of excitatory transmitter release.[215]

Whatever the underlying mechanism, the clinical syndrome of ADNFLE is known to respond well to carbamazepine and topiramate. Carbamazepine is a noncompetitive inhibitor of nicotinic receptors that blocks acetylcholine-evoked currents at concentrations in the therapeutic range. The mutant receptors in ADNFLE are about threefold more sensitive to this action of carbamazepine than are wild-type receptors.[216] It seems plausible that the enhanced action on nicotinic receptors contributes to the anticonvulsant effect of carbamazepine in ADNFLE. These studies also raise the possibility that an interaction with nicotinic receptors could contribute to the action of carbamazepine in other epilepsy syndromes.

Glycine receptors. The glycine receptors are cys-loop ligand-gated Cl^- channels that are structurally and functionally similar to $GABA_A$ receptors and play an important inhibitory role in the spinal cord and brain stem. Like other members of the cys-loop superfamily, glycine receptors are pentameric in structure and are composed of α and β subunits. Multiple genes encoding the α subunits have been identified (α1–4 in the mouse and α1–3 in humans), whereas there is only one β subunit gene.[217,218] The β subunit anchors the receptor to the cytoplasmic protein gephyrin.

Several endogenous ligands including glycine, taurine, or betaine are able to open glycine receptors. Glycinergic synapses exist in brain stem nuclei and the cerebellum, but not in the cortex or hippocampus. Nonsynaptic glycine receptors are found on hippocampal pyramidal neurons and may provide a tonic current in response to ambient taurine and glycine.[219] Glycine receptors are positively modulated by volatile anesthetics, n-alcohols (including ethanol), and chloral derivatives.[220]

Strychnine is a powerful selective antagonist of glycine receptors that binds selectively to glycinergic synapses. Strychnine induces convulsions with fierce contractions of skeletal musculature, giving an arched back and risus sardonicus. Seizure activity does not spread to the forebrain or cortex, so that consciousness is maintained during strychnine convulsions. No naturally occurring epilepsy syndrome in humans has this "spinal" characteristic, and no human epilepsy syndromes have been associated with glycine receptors. However, a variety of mutations in the α1 subunit (which reduces the glycine-receptor Cl^- current) have been found in the autosomal dominant disorder hyperekplexia (startle disease) in humans, mice, and cattle. Glycine receptors lack a functional role in the forebrain, so they are unlikely to be a useful AED target.

Ionotropic glutamate receptors

The ionotropic glutamate receptors are a superfamily of ligand-gated cation channels that encompass three receptor families identified by the agonists that selectively activate them: AMPA, kainate, and NMDA.[221–223] These channels mediate most of the fast excitatory transmission in the central nervous system and are thus involved in all brain functions. Their excessive activation plays a large role in epileptic phenomena, including the paroxysmal depolarization shift (the single-cell correlate of interictal spikes) and seizure discharges.

Neurotransmitter glutamate serves to activate all ionotropic glutamate receptors at synapses. However, NMDA receptors require the presence of a coagonist, either glycine or D-serine.[224] The coagonists are not released during synaptic transmission. Rather, their concentrations are regulated by various processes. For example, the levels of glycine are maintained at levels below those that saturate NMDA receptors by glycine transporter-1 (GlyT1), a Na^+/Cl^--dependent carrier molecule that exists in several isoforms (a–e) in glial and neuronal cell membranes. GlyT1 can operate bidirectionally: decreasing synaptic glycine concentrations when operating in the forward direction and releasing glycine from glial cells when operating in reverse mode. GlyT1b is found in astrocytes and colocalizes with NMDA receptors in the forebrain; it can powerfully modulate NMDA receptor-mediated responses.[225] D-Serine production is regulated by its synthesizing enzyme serine racemase.[226] The var-

TABLE 6. *Ionotropic Glutamate Receptors: Subtype Pharmacology and Actions of AEDs*

Receptor Subunits, by Family	Agonists	Competitive Antagonists	Allosteric Antagonists	Channel Blockers	AED Actions
			AMPA		
GluR1 (GluR-A), GluR2 (GluR-B), GluR3 (GluR-C), GluR4 (GluR-D)	AMPA, ACPA, quisqualate, willardine, kainate	NBQX, NS1209, Ro 48-8587, zonampanel (YM872), ZK-20075	GYKI 52466, talampanel		Phenobarbital
			Kainate		
GluR5, GluR6, GluR7, KA1, KA2	Kainate, domoate, SYM 2081, (*S*)-ATPA, iodo-willardine	NS 102, NBQX (*nonselective*); LY 293558, LY 382884, UBP196, UBP310 (*GluR5 selective*)	NS 3763		Topiramate (*GluR5 selective*)
			NMDA		
NR1, NR2A, NR2B, NR2C, NR2D, NR3A, NR3B	Glycine, D-serine, D-cycloserine, NMDA, L-aspartate, homocysteate	Kynurenic acid, L-701-324, licostinel, gavestinel, MDL 105,519 (*glycine site*); AP7, D-CPPene, CGS 19755 (*glutamate site*); PPDA (*NR2C/NR2D selective*)	Ifenprodil, traxoprodil (*NR2B selective*)	Phencyclidine, MK-801 (dizocilpine), aptiganel (*dissociative anesthetic-like*); Memantine, ADCI (*low affinity*)	Felbamate, conantokin G (*NR2B selective*); remacemide (*des*-glycine metabolite)

ious regulatory mechanisms for NMDA receptors related to coagonists suggest a variety of strategies for modulating NMDA receptor function.

Structure of ionotropic glutamate receptors. Ionotropic glutamate receptors are tetrameric structures, usually composed of more than one type of subunit.[222] Each of the families has a specific set of subunits: four for the AMPA receptors, five for the kainate receptors, and seven for the NMDA receptors (TABLE 6). Alternative splicing and RNA editing of the subunits further contribute to diversity in channel properties. Each subunit has an extracellular amino-terminal domain (ATD), three transmembrane segments (M1–M3), and an intracellular carboxy terminal (FIG. 5).

The most remarkable structural feature of ionotropic glutamate receptor subunits is the "venus flytrap" agonist-binding site formed by two peptide loops, S1 linking the ATD with M1 and S2 linking M2 and M3.[227] Between M1 and M2, there is a re-entrant pore loop that provides the ion-selective filter that, in contrast to the voltage-gated ion channels, is on the inner aspect of the membrane.

All ionotropic glutamate receptors are permeable to Na^+ and K^+, but differ in Ca^{2+} permeability. In particular, NMDA receptors have high Ca^{2+} permeability. AMPA subunits exist in two forms, "flip" and "flop," produced by alternative splicing. Most AMPA receptors are Ca^{2+}-impermeable, unless they lack edited GluR2 (GluR-B) subunits, in which case they are Ca^{2+}-permeable. Such Ca^{2+} permeable AMPA receptors show inward rectification due to voltage-dependent block of the pore by polyamines. The biosynthesis of the GluR2 subunits that compose Ca^{2+}-impermeable AMPA receptors occurs by the unique mechanism of pre-mRNA editing, in which a codon for glutamine is transformed by ADAR2 (an adenosine deaminase that acts on RNA) to one coding for arginine at the Q/R site.

The crystal structures of NMDA, AMPA, and kainate receptors have been determined, and the specific features governing agonist and antagonist binding have been elucidated.[227–229] This improves the prospect of designing novel molecular structures functioning as subunit-specific antagonists. Progress along these lines is already evident, as shown, for example, by the identification of PPDA [(2*S**,3*R**)-1-(phenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid] as a highly selective antagonist of NMDA receptors composed of NR2C/NR2A-D subunits.[230]

NMDA receptors. All NMDA receptors contain an NR1 subunit, which exists as eight splice variants.[221]

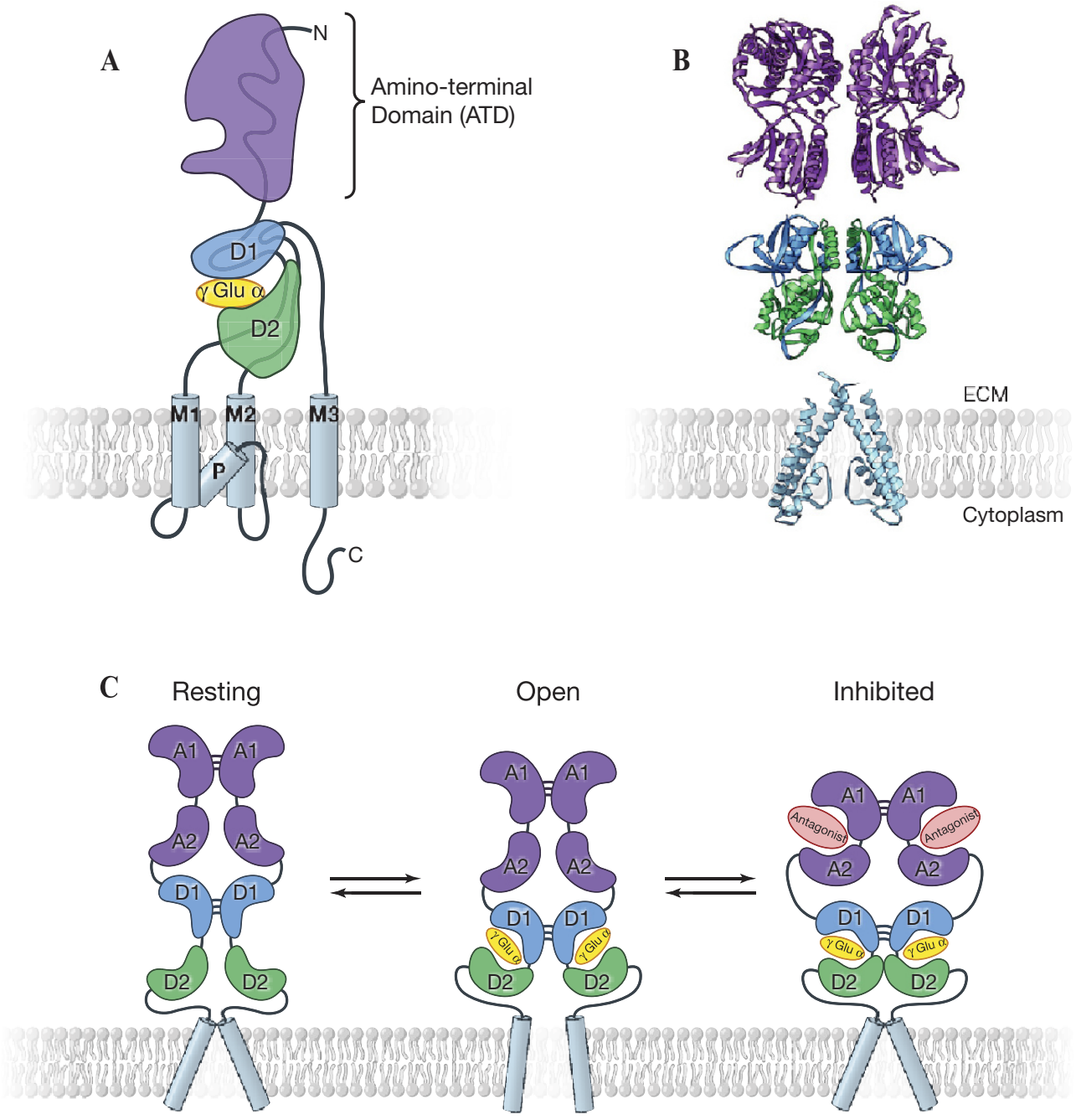

FIG. 5. Ionotropic glutamate receptors. **A:** Membrane topology of the ionotropic glutamate receptors, showing the extracellular amino terminal domain (ATD) with the "venus flytrap" ligand binding structure that mechanically opens the pore. The extracellular aminoterminal linked to M1 interacts with the extracellular loop connecting M2 and M3 to form the two globular elements of the venus flytrap. **B:** Dimeric model of the glutamate ionotropic receptor. The functional receptor is thought to be a double dimer. For the NMDA receptor, the dimer includes one NR1 subunit, binding glycine or D-serine, and one NR2A-D subunit, binding glutamate or aspartate. **C:** Hypothetical model of binding of allosteric inhibitors such as Zn^{+} and ifenprodil to the amino-terminal domain. Modified from Mayer,[227] with permission.

They additionally contain one or more of the Nr2a–D group (and may contain an NR3A or NR3B). There are regional differences in the brain in the expression of the NR2A–D subunits. The NR1 subunit binds glycine, not glutamate; the various NR2 subunits bind glutamate. The binding of two glycine and two glutamate molecules is required for channel opening. It is thought that the functional channel comprises two dimers, one composed of NR1 subunits and one with NR2 subunits (FIG. 5).

An important special functional property of NMDA receptors is that they are blocked in a voltage-dependent fashion by Mg^{2+}, such that the open channels are largely

impermeable to ion flow at negative membrane potentials (near the resting potential), due to retention of Mg^{2+} in the channel pore. Depolarization of the membrane (for example, by activation of neighboring AMPA receptors) overcomes the Mg^{2+} block, allowing for current flow. The requirement for membrane depolarization in addition to glutamate signaling is considered to be the coincidence detector of Hebbian learning; it may also be important in epileptogenesis. A further element of coincidence detection may be provided by astrocytes that respond to synaptic glutamate release by releasing D-serine (which, as already noted, serves as a coagonist of NMDA receptors).[226]

AMPA receptors. AMPA receptors are composed of four types of subunits, designated GluR1–4 (alternatively GluRA–D), which combine to form tetramers. Most AMPA receptors are either homotetramers of GluR1 or GluR4, or symmetric "dimer of dimers" of GluR2/3 and either GluR1 or GluR4. Each AMPA receptor subunit has a binding site for agonist (such as glutamate); the channel opens when two or more sites are occupied.

AMPA receptors serve as the primary mediators of fast excitatory neurotransmission in the mammalian central nervous system, and changes in their cellular expression underlie forms of synaptic plasticity. Systemic or intracerebroventricular administration of AMPA elicits robust seizure activity, demonstrating a role for AMPA receptors in triggering or mediating seizures. Similarly, pharmacological antagonists of AMPA receptors are protective in diverse animal seizure models, raising the possibility that AMPA receptor antagonists could be useful in epilepsy therapy. Although AMPA receptors appear to be critical for the expression of kindled seizures, they are not required for kindling development as are NMDA receptors. In kindling models, consequently, AMPA receptor antagonists do not have the antiepileptogenic properties of NMDA receptor antagonists.[231,232] In epileptogenesis models in which a susceptibility to spontaneous seizures is triggered by status epilepticus, however, AMPA receptor antagonists can have antiepileptogenic activity, if only by virtue of their ability to powerfully suppress the seizures.[233]

Genetics. There is little evidence for spontaneous mutations involving glutamate receptors in epilepsy syndromes in human or mouse. There has been an association with juvenile absence epilepsy of a nine-repeat allele of a tetranucleotide repeat polymorphism in a noncoding region of the GluR5 receptor gene (*GRIK1*).[234] A more recent study, however, failed to confirm that mutations in *GRIK1* affecting the structure of GluR5 are related to this idiopathic epilepsy syndrome.[235]

Studies with genetically engineered mice have shown that alterations in GluR2 editing that cause AMPA receptors to be Ca^{2+} permeable lead to seizures. Thus, a GluR2-editing-deficient mouse [specifically, with an induced mutation at a single codon that prevents editing of GluR2(Q) to GluR2(R)] exhibits severe spontaneous seizures beginning around P14 that usually prove lethal by P20–25.[236] Moreover, it was possible to show that the vulnerability to seizures induced by GluR2(Q) does not require the defect to be present during development, in that switching on of GluR2(Q) in adult mice also leads to a seizure phenotype.[237]

The precise mechanism initiating the seizures remains undefined, but involves both hippocampal and nonhippocampal neurons, because switching on GluR2(Q) in hippocampal neurons alone does not lead to seizures. A role for genetic alterations in Q/R site editing of AMPA has not been confirmed by studies of the editing status of receptors in brain tissue from patients with temporal lobe epilepsy.[238]

Drug development. Because of the role of glutamate in the pathophysiology of seizures and the empirical evidence that ionotropic glutamate receptor antagonists are protective in various animal seizure models, substantial effort has been devoted toward the development of such antagonists for epilepsy therapy.[20] To date, the results of this development effort have not been encouraging. No drug designed specifically as a glutamate receptor antagonist has been approved for marketing, although several marketed drugs appear to interact with ionotropic glutamate receptors, and this interaction could contribute to the therapeutic efficacy. The pharmacology of ionotropic glutamate receptors as AED targets has been reviewed previously,[20] and only selected aspects will be considered here.

In the 1970s, as the existence of pharmacologically distinct subtypes of glutamate receptors became apparent through the use of selective agonists, a medicinal chemistry program by J.C. Watkins at the University of Bristol, UK, began to identify subtype-selective antagonists that have led to the development of a diverse repertoire of experimental tools[239] (TABLE 5). In 1982, the ability of competitive NMDA receptor antagonists to block seizures in rodent epilepsy models was identified.[240] Subsequently, noncompetitive NMDA antagonists such as MK-801 (dizocilpine), glycine site antagonists (such as HA-966) and NR2B site antagonists (such as ifenprodil) were all shown to possess protective activity in some rodent models.[241] In models of generalized seizures, competitive NMDA antagonists appeared the most promising (in terms of efficacy and therapeutic ratio); in kindled seizures in rodents, they were less effective, and side effects were enhanced relative to nonkindled animals, apparently as a result of altered properties of the NMDA receptors.[242] In a preliminary add-on trial in complex partial seizures, toxicity but no efficacy was reported for the competitive antagonist D-CPPene.[243]

An alternative approach to blocking NMDA receptors

is provided by agents that bind to NMDA receptors within the ion channel conduction pore. Such so-called uncompetitive antagonists include the high-affinity blockers MK-801, phencyclidine, and aptiganel, which are unlikely to be of clinical utility because they are associated with neurobehavioral side effects and are potentially neurotoxic.[244] (Note, however, that the neuronal vacuolization and necrosis observed with MK-801 and also memantine, in rodent models, have not been detected in animals with larger brains, including primates.)

Low-affinity open-channel blockers (such as memantine, dextrorphan, ADCI [5-aminocarbonyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine], and the active *des*-glycine metabolite of remacemide) have a reduced propensity for neurological and behavioral side effects, and may have limited or no neurotoxicity.[245–247] ADCI and remacemide seem to owe their broad-spectrum activity in animal seizure models to a combination of NMDA receptor antagonism and an action on voltage-gated Na^+ channels.[248]

AMPA receptor antagonists, which are anticonvulsant in a broad range of rodent animal models and also in photosensitive baboon, may have greater potential clinical utility than do the NMDA antagonists.[25,249–253] In fact, they may be active in *in vitro* epilepsy models even when NMDA receptor antagonists are not.[254] AMPA receptor antagonists can suppress status epilepticus-like activity in some animal models.[233]

Current therapy for convulsive status epilepticus is less than optimal. Patients failing initial therapy often require general anesthesia with agents such as pentobarbital. These anesthetic agents may depress myocardial function and reduce blood pressure, resulting in a high incidence of systemic complications that can lead to permanent functional impairment or death. AMPA receptor antagonists have the potential to stop seizures more effectively and safely than do current second-line agents, without the cardiovascular side effects. In addition, they may confer neuroprotection by blocking glutamate-induced excitotoxicity, which could diminish the brain damage and neurological morbidity typically associated with status epilepticus.

The first selective AMPA receptor antagonists to be identified were quinoxalinedione derivatives (such as NBQX), which act as competitive antagonists to inhibit glutamate gating of AMPA receptors.[20] The early quinoxalinediones had poor solubility, such that they precipitated in the kidney, leading to crystalluria and nephrotoxicity; however, newer quinoxalinedione analogs and nonquinoxalinediones, such as zonampanel (YM872) and ZK-200775, have largely overcome this problem.

The high-affinity, water-soluble competitive antagonist NS1209 (which blocks AMPA and GluR5 kainate receptors)[255] is effective in animal models of status epilepticus and prevents the development of spontaneous seizures. It has undergone clinical evaluation in humans for the treatment of refractory status epilepticus.[25] Because NS1209 is not orally bioavailable, it is administered intravenously.

Subsequent to the discovery of competitive AMPA receptor antagonists, a series of 2,3-benzodiazepines were described that acted as selective allosteric (noncompetitive) antagonists of AMPA receptors.[256–258] These agents inhibit AMPA receptor currents at low concentrations, but are less potent on kainate receptors and are inactive at NMDA receptors. They are effective orally and parenterally in diverse animal seizure models, except in the WAG/Rij rat model of absence epilepsy, in which they perform only poorly.[259,260] Domain-swapping and site-directed mutagenesis has demonstrated that the binding site for such noncompetitive antagonists is within peptide segments of the AMPA receptor subunits that link the transmembrane spanning regions and the ligand binding core.[261] These regions of the receptors are believed to transduce agonist binding into channel gating. Binding of the noncompetitive antagonists is likely to disrupt this transduction process. One 2,3-benzodiazepine, talampanel (GYKI 53773; LY300164), has shown efficacy in clinical trials.[262,263]

Nonetheless, it is unclear whether AMPA receptor antagonists will be tolerable at therapeutic doses. In animal models, such agents generally cause neurological impairment at doses near those that confer seizure protection. In humans, acute treatment with AMPA receptor antagonists results in sedation, dizziness, and ataxia. Although not an issue in the emergency treatment of status epilepticus, these side effects could be problematic in the use of AMPA receptor antagonists for chronic epilepsy therapy. Whereas NMDA receptor antagonists can produce schizophrenia-like symptoms, perceptual alterations, and cognitive and memory impairment, AMPA receptor antagonists have no such psychoactive properties.

Three marketed AEDs have been shown to interact with glutamate receptors. Phenobarbital modestly decreases the depolarizing or excitotoxic action of AMPA and kainate at concentrations similar to those at which it potentiates GABA, but this effect seems unlikely to be contributing significantly to the sedative or anticonvulsant actions of barbiturates.[264,265]

Among the various cellular actions of topiramate, effects on kainate and AMPA receptors may be of significance. Thus, topiramate has been reported to block kainate-induced currents in cultured hippocampal neurons,[266] and recent evidence has noted that it acts specifically on GluR5 kainate receptors and with lower potency on AMPA receptors.[267–269] Topiramate may act indirectly by altering the phosphorylation state of its targets.[29,270]

Like topiramate, felbamate has several different phar-

macological actions that have been proposed as contributing to its clinical efficacy. Among these is a specific inhibitory effect on NMDA receptors.[271] Studies with recombinant NMDA receptors have indicated that felbamate preferentially blocks NMDA receptors containing NR2B subunits.[272,273] The drug acts as an allosteric modulator of channel gating,[274] with a preferential action to stabilize the inactivated state so as to produce a use-dependent block.[275] This use-dependent action may selectively inhibit NMDA receptors that are excessively activated as is believed to occur during seizure discharges.

ACID-SENSING ION CHANNELS

Acid-sensing ion channels are a family of proton-gated cation channels related to the degenerin/epithelial Na^+ channels, which have diverse functions in metazoan cells.[276] Six isoforms (ASIC1–6) have been cloned; these are widely expressed as homomeric or heteromeric channels in the central and peripheral nervous system.[277] ASICs in sensory neurons are believed to be involved in nociception when injury or inflammation causes acidification. The functional roles of ASICs in the brain are less well understood.

It has been suggested that protons released during high-frequency stimulation of excitatory synapses activate ASICs to cause postsynaptic depolarization.[278] Among the consequences of this depolarization may be reduction in the Mg^{2+} block of NMDA receptors, which would promote epileptic activity. Thus, pharmacological inhibition of ASICs might reduce excitatatory synaptic transmission under these circumstances and have anticonvulsant actions. Moreover, acidification occurs during intense seizure activity, which could activate ASICs and contribute to seizure-induced brain damage, particularly because many ASICs are Ca^{2+} permeable.[279] ASIC antagonists might minimize these adverse consequences of seizures.

ASIC disruption in mice had no adverse developmental or behavioral consequences, and did not affect ordinary synaptic transmission.[278] Thus, ASIC antagonists may have fewer adverse effects than other antagonists of excitatory neurotransmission. At present, no selective ASIC antagonists are available to test the role of ASICs as anticonvulsant targets; however, the potassium-sparing diuretic amiloride does act as an ASIC antagonist and it appears to have anticonvulsant properties.[280] The peripheral cardiotoxicity of amiloride caused by its hyperkalemic action can be reduced by a liposome encapsulation. Status epilepticus has been shown to cause downregulation of ASIC mRNA (types 2b and 1a) in the hippocampus, raising the possibility that resistance to ASIC antagonists could occur under some circumstances.[281]

G-PROTEIN-COUPLED RECEPTORS

G-protein-coupled receptors (GPCRs) are the largest family of receptors with as many as 1000 members, including many that have been identified from the sequencing of the human genome but are of unknown function. It is claimed that they comprise the largest set of therapeutic drug targets for known medicinal agents.[282,283]

All GPCRs have an extracellular N-terminal, an intracellular C-terminal, and seven α-helical transmembrane segments. They are divided into three main classes (1–3 or A–C). We shall discuss only class 3/C, which includes the metabotropic glutamate receptors (mGluRs) and the $GABA_B$ receptors, which play important roles in controlling excitability at glutamatergic and GABAergic synapses.[284,285] These two types of receptors share several features, including their functional expression as dimers, although the mGluRs seem to be mostly homomeric, whereas functional $GABA_B$ receptors are heterodimers.

Many peptide neurotransmitters, such as neuropeptide Y, galanin, somatostatin, and the enkephalins and endorphins, which act at GPCRs, are involved in epileptic phenomena. A very extensive literature describes how compounds acting on their receptors influence seizure susceptibility. No established AED is thought to act via these receptors. For reasons of space, we omit further discussion of peptide neurotransmitter receptors.

Metabotropic glutamate receptors

The metabotropic glutamate metabotropic receptors are a family of eight G-protein-linked receptors that fall into three groups defined by their sequence homology, second messenger effects, and common pharmacology[286,287] (TABLE 7). They have seven transmembrane segments, with an intracellular loop between segments 3 and 4 that binds the G protein and a large "venus flytrap" extracellular element that is homologous with bacterial periplasmic proteins. In neurons, group I receptors are primarily located postsynaptically and have excitatory effects mediated by several ion channels. Group II and III receptors are located presynaptically, where their activation reduces transmitter release through inhibition of voltage-gated Ca^{2+} channels or potentiation of inwardly rectifying K^+ channels.[288] Astrocytes also express mGluR3 and mGluR5. mGluR2, a member of group II, is located some distance from the synaptic cleft on the axon and has high affinity for glutamate, whereas mGluR7, a member of group III, is located within the cleft and has a low glutamate affinity.

There is little evidence to indicate that genetic alterations of mGluRs play a role in the pathogenesis of epilepsy. Spontaneous mutations causing epilepsy are not known in mice or in humans; however, mice in which GluR7 has been deleted by gene targeting show an in-

TABLE 7. *Metabotropic Glutamate Receptors: Neuronal Localization, Genetics, and Pharmacology in Relation to Seizures*

Receptor	G protein	Location	Genetics	Agonists	Antagonists
			Group I		
mGluR1	G_q linked to phospholipase C	Postsynaptic, perisynaptic		DHPG (*convulsant*)	LY367385, LY456236, AIDA (*anticonvulsant*)
mGluR5				DHPG, CHPG (*convulsant*)	MPEP, SIB-1893 (*anticonvulsant*)
			Group II		
mGluR2, mGluR3	$G_{i/o}$ linked to adenyl cyclase and voltage-gated K^+ and Ca^{2+} channels	Presynaptic ("axonal")		*LY354740, LY389795, LY379268* (*anticonvulsant*)	2α-ethylglutamate (*convulsant*)
			Group III		
mGluR4, mGluR7, mGluR8	$G_{i/o}$ linked to adenyl cyclase and voltage-gated K^+ and Ca^{2+} channels	Presynaptic (synaptic cleft)	mGluR7 knockout → increased seizure susceptibility	2-AP4, L-SOP (*mixed activity*); ACPT-1, (*R,S*)-PPG, (*S*)-3,4-DCPG (*anticonvulsant*)	*MAP-4*, *MSOP*, *MPPG*, (*convulsant*); *MCPA* (*anticonvulsant*)

creased susceptibility to seizures and increased cortical excitability.[289]

Acquired forms of epilepsy show marked changes in the expression and function of mGluRs. In the kindling model of epilepsy, there is upregulation of group I receptors that can be demonstrated by biochemical or electrophysiological functional assays.[290,291] There is also upregulation of mGluR5 in the hippocampus of patients with complex partial seizures.[292] In contrast, downregulation of hippocampal group II mGluRs was seen in chronically epileptic rats that experienced pilocarpine-induced status epilepticus.[293] A downregulation of group III mGluRs has also been reported in hippocampal slices from kindled rats[294] and from patients with complex partial seizures.[295] The possible role of these changes in mediating the epileptic state has not been well defined, but is potentially important, in that such changes all tend to enhance excitability.

The variety of compounds that show some selectivity as agonists or antagonists for the different subtypes of glutamate metabotropic receptor has been reviewed previously.[296] Much attention has been paid recently to compounds that act as allosteric blockers or potentiators of these receptors, because they have the dual advantage of greater specificity of action and lesser susceptibility to adaptive compensation.[297–299] Studies in animal models of epilepsy have provided a broadly consistent pattern of effects of selective agonists, antagonists, and allosterically acting compounds—although there are some puzzling anomalies.[300,301] Group I agonists such as DHPG [(*R,S*)-3,5-dihydroxyphenylglycine] and CHPG (2-chloro-5-hydroxyphenylglycine) are convulsant. Group I antagonists such as AIDA (1-aminoindan-1,5-dicarboxylic acid), LY367385, LY456236, MPEP [2-methyl-6-(phenylethynyl)-pyridine], and SIB-1893 are anticonvulsant in a variety of animal models, including generalized convulsive and absence-like seizures, and in models of complex partial seizures (6 Hz, MES, and amygdala-kindled seizures).[300,302–304] A lack of anticonvulsant efficacy has, however, been reported for the potent mGluR1 antagonist EMQMCM [3-ethyl-2-methyl-quinolin-6-yl-(4-methoxy-cyclohexyl)-methanone methanesulfonate] and the potent mGluR5 antagonist MTEP {[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine} in the 6-Hz electroshock model and the amygdala-kindled rat,[305] suggesting that group I antagonists are unlikely to be effective in the largest group of pharmacotherapy-resistant patients.

Group II agonists are anticonvulsant in a variety of rodent models, including those for absence epilepsy.[300,301] Some group II antagonists, such as 2α-ethylglutamate, are proconvulsant, but others, such as APICA [(*R,S*)-1-amino-5-phosphonoindan-1-carboxylic acid] are not. A potent positive allosteric modulator of mGluR2, biphenyl-indanone A, has recently been described and characterized.[306] It has antipsychotic- and anxiolytic-like effects in animal models, but its actions in seizure models have not yet been reported.

The classical group III agonists, 2-AP4 [(L)(+)-2-amino-4-phosphonobutyric acid] and L-SOP (L-serine-*O*-phosphate), show mixed anti- and proconvulsant effects in rodents. More recently identified group III agonists such as ACPT-1 [(*1S,3R,4S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid], (*R,S*)-PPG [(*R,S*)-4-phosphonophenylglycine], and (*S*)-3,4-DCPG [(*S*)-3,4-dicarboxyphenylglycine] show clear anticonvulsant effects.[307]

Most group III antagonists, such as the methyl derivatives of 2-AP4 (MAP-4), L-SOP (MSOP), and (*R,S*)-PPG (MPPG), are convulsant. MCPA [(*S*)-α-methyl-3-carboxyphenylalanine], which is a poor group II antagonist in electrophysiological assays but blocks the action of L-AP4 stimulated cAMP production, is anticonvulsant. Selective allosteric agonist (also known as positive modulators) of mGluR4 (PHCCC) and mGluR7 (AMN082) have recently been described, but their actions in seizure models have not been reported.[299,308] Metabotropic glutamate receptors clearly are significant molecular targets for AED development. Agents acting presynaptically to reduce glutamate release may be most effective in combination with AMPA or NMDA receptor antagonists.[309]

$GABA_B$ receptors

$GABA_B$ receptors were originally identified as the receptors mediating the bicuculline-insensitive, Cl^--independent inhibitory effects of GABA and baclofen on sympathetic ganglion neurons and presynaptic terminals.[218,310] They were subsequently shown to play an important role in mediating the actions of GABA in the central nervous system. $GABA_B$ receptors are expressed presynaptically at GABAergic and glutamatergic synapses, where they act to decrease neurotransmitter release by reducing Ca^{2+} entry. $GABA_B$ receptors are also expressed postsynaptically at GABAergic synapses, where they produce a late hyperpolarization in response to synaptically released GABA by enhancing K^+ permeability through GIRKs ($K_{ir}3.x$).

Like metabotropic glutamate receptors, $GABA_B$ receptors are G-protein-coupled receptors and they share the "venus flytrap" extracellular ligand-binding domain. The functional receptor is a heteromeric dimer made up of a $GABA_{B1}$ and $GABA_{B2}$ subunit[311,312] (FIG. 6). $GABA_{B1}$ binds GABA, and $GABA_{B2}$ is required for the heteromer to reach the cell surface and plays a key role in G protein coupling. In the thalamus, $GABA_B$ receptors, activated by strong firing of thalamic reticular nucleus (TRN) neurons, contribute to the generation of rhythmic activity and are required for oscillations at 3–4 Hz, which is similar to the spike-and-wave seizure frequency range in humans.[313] In the GAERS model of absence epilepsy, there is an upregulation of both $GABA_{B1}$ and $GABA_{B2}$ protein in corticothalamic circuits, which may contribute to the seizure phenotype.[314]

Baclofen, the classical $GABA_B$ receptor agonist, is selective for $GABA_B$ receptors and does not activate $GABA_A$ receptors. Several analogs have been developed as selective agonists [APPA (3-aminopropyl-phosphinic acid)] and antagonists (phaclofen, saclofen, CGP 62349).[315] These bind to the $GABA_{B2}$ subunit but their affinity is much greater for the native dimer. Recently, CGP7930 has been identified as a positive allosteric modulator, which binds to the $GABA_{B2}$ subunit and can activate the receptor in the absence of GABA.[316] $GABA_B$ agonists such as baclofen promote spike-and-wave discharges, and antagonists such as phaclofen suppress them in rodent absence epilepsy models.[317] $GABA_B$ receptors represent a promising target for the development of antiabsence agents.

NEUROTRANSMITTER TRANSPORTERS

There are three neurotransmitter transporter families.[318] Vesicular transporters serve to transport specific neurotransmitters into synaptic vesicles, including glutamate (VGLUT1–3), GABA and glycine (VGAT/VIAAT), acetylcholine (VAC), and biogenic amines (catecholamines and serotonin) (VMAT). Changes in the function or expression of these transporters can modify the quantal size of synaptic potentials.[319] Plasma membrane transporters regulate the activity of neurotransmitters including GABA and biogenic amines by sequestering them into cells after they are released from nerve terminals. They couple transmembrane movement of Na^+ and Cl^- (and in some systems, K^+) to the reuptake of the neurotransmitter. The third family transports glutamate and related substances, including D- and L-aspartate, across the plasma membrane and is coupled to Na^+.

Both vesicular and plasma membrane transporters move transmitters uphill, against a concentration gradient, and require energy from the hydrolysis of ATP. At the plasma membrane, the energy is utilized by (Na^+,K^+)-ATPase in generating ion gradients that provide the driving force for neurotransmitter uptake. In the case of the vesicular transporters, ATP is hydrolyzed by a H^+ pump (V-type H^+-ATPase) that acidifies the vesicle interior, generating a proton gradient that drives transport. Neurotransmitter transporters engage in complex functional interactions with themselves and with many other proteins (e.g., syntaxin 1A), modifying their localization and function. Neurotransmitter transporters are electrogenic because of the ions that are coupled to transport of the solute. They generate currents that exceed predictions, and are believed to have channel-like properties in addition to the transporter function.[320]

Plasma membrane GABA transporters

In mammals, four transporters that convey GABA and some related amino acids across the plasma membrane of neurons and astrocytes have been cloned and functionally expressed[321,322] (TABLE 8). Their nomenclature originally differed in human and rat and in the mouse because a betaine transporter identified in dog kidney and named BGT-1 (betaine/GABA transporter 1 protein) was subsequently shown to be closely homologous to a GABA transporter in the human brain. The four transporters are as follows, with the corresponding human and

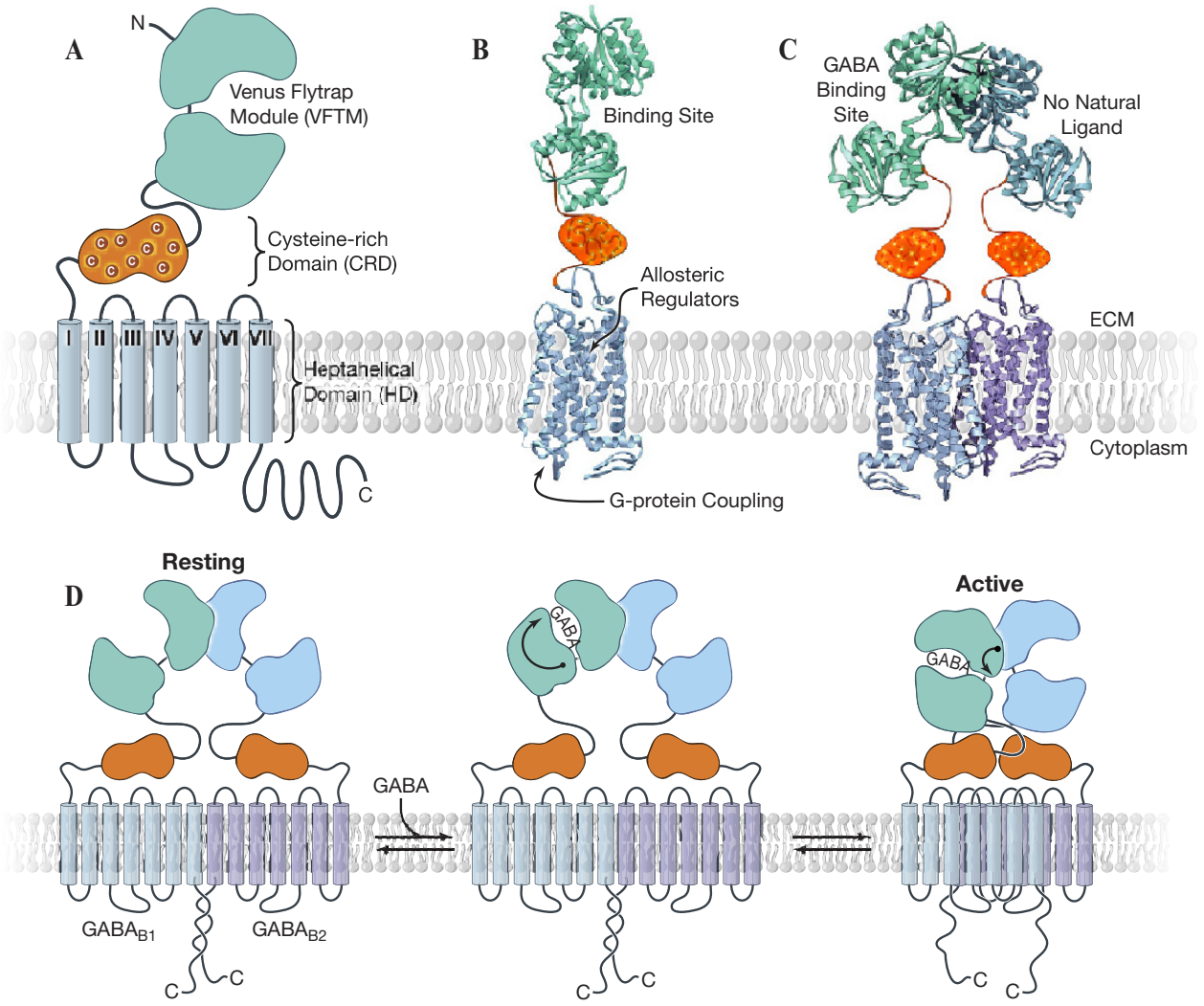

FIG. 6. G-protein-coupled (metabotropic) receptors. **A:** Membrane topology of class 3/C G-protein-coupled receptors, showing intracellular C-terminal, extracellular N-terminal, seven transmembrane helices, the cysteine-rich domain and the "venus flytrap" module.[284–286] **B:** Ribbon diagram corresponding to A showing sites of interaction with agonists, allosteric regulators and G-proteins. **C:** Ribbon diagram showing the assembly of a $GABA_{B1}$ and a $GABA_{B2}$ subunit to form the dimeric $GABA_B$ receptor. **D:** Diagram showing the conformational change following agonist binding to the venus flytrap module of the B1 subunit of the dimeric $GABA_B$ receptor, with transition from the resting to the active state.[312]

murine gene symbols in italics (all capital letters for human genes, an initial capital for murine genes): GAT-1 (human *SLC6A1*, alias *GAT1*; murine *Slc6a1*, alias *Gat1*, *Gabt*); BGT-1 (human *SLC6A12*, alias *BGT-1*; murine *Slc6a12*, alias *Gat2*, *Gabt2*), GAT-3 (human *SLC6A11*, alias *GAT3;* murine *Slc6a11*, alias *Gat3, Gatbt3, Gat4,*

TABLE 8. *GABA Transporters: Cellular and Regional Localization, Pharmacological Inhibitors, and Functional Roles*

Transporter	Cellular and Regional Localization	Inhibitors	Function
GAT-1 (human, rat, mouse)	"Neuronal" (forebrain), nerve terminals; also in astrocytes	SKF-89976, tiagabine, LU-32-176B, EF1502, NO-711	Shapes phasic inhibition
BGT-1 (human), GAT-2 (mouse)		EF1502	
GAT-3 (human, rat), GAT-4 (mouse)	"Glial"; possibly also in cortical neurons	SNAP-5114	With GAT-1 controls tonic inhibition
GAT-2 (human, rat), GAT-3 (mouse)	Glia; neurons (extrasynaptic)	SNAP-5114	

Gabt4), and GAT-2 (human *SLC6A13*, alias *GAT2*; murine *Slc6a13*, alias *Gat3*, *Gabt3*). GABA transporters are structurally homologous to the transporters for glycine, taurine, and the monoamines, which have 12 putative transmembrane helical elements.

The primary function of GABA transporters is to remove synaptically released GABA, thereby limiting or terminating its inhibitory action.[323] Reuptake into terminals permits immediate recycling by vesicular uptake, whereas reuptake into astrocytes leads to metabolism via GABA-transaminase and succinic semialdehyde dehydrogenase. Studies with subtype-selective GABA-transporter inhibitors indicate that GAT-1 influences the shape of phasic responses, prolonging IPSCs in cortex and hippocampus.[324] Inhibition of GAT-2 or GAT-3 is most effective when combined with GAT-1 inhibition.

Early structure–activity studies of inhibitors of GABA uptake using neuronal and astrocytic cultures suggested that glial and neuronal uptake could be differentially targeted.[325] These studies furthermore indicated that compounds inhibiting astrocytic uptake were anticonvulsant, but those acting on neuronal uptake were not.[9,326] Cloning of the transporters, the demonstration that GAT-1 is the predominant transporter in astrocytes and neurons, and the demonstration that tiagabine affects neuronal and astrocytic uptake to a comparable degree, have thrown this interpretation into doubt.

Tiagabine, which is highly selective for GAT-1, has a distinctive spectrum of activity in preclinical models of epilepsy that is consistent with the selective expression of GAT-1 in cortex and limbic structures, but not in hindbrain and brainstem. Thus, the drug is highly protective against fully kindled seizures in rodents, but is only weakly effective against MES seizures, which are generated in the brainstem.[327] In addition, tiagabine exacerbates spike-and-wave discharges in some models of absence epilepsy, prossibly due to enhanced action of GABA at $GABA_B$ receptors.[328] Acute studies in hippocampal slices show that tiagabine prolongs inhibitory synaptic potentials.[324] However, in GAT-1 knockout mice, IPSCs are not potentiated, phasic GABA release is downregulated, and GABA-mediated tonic conductance is potentiated.[329] Thus, the long-term antiepileptic action of tiagabine may be mediated by potentiation of the tonic inhibitory effect of GABA in the forebrain rather than enhancement of phasic inhibition.[330]

Studies with a novel GABA-transporter inhibitor, EF1502, that potently inhibits GAT-1 and GAT-2 (BGT-1)—but not GAT-3 and GAT-4—indicate that inhibition of GAT-2 potentiates the anticonvulsant activity of GAT-1.[331,332] GAT-3 can be selectively inhibited with SNAP-5114, which enhances GABA-mediated synaptic inhibition in the neocortex, indicating that GAT-3 plays a role in this brain region.[333] Studies in resected tissue from patients with complex partial seizures and hippocampal sclerosis indicate that the expression of GAT-1 is decreased, but that of GAT-3 may be increased, possibly explaining prolonged IPSCs and increased excitability reported in electrophysiological studies with this tissue.[334,335] Although the selective GAT-1 inhibitor tiagabine is a well recognized AED, GAT-2 and GAT-3 can now also be considered as potential targets.

The plasma membrane GABA transporters release GABA into the extracellular space when transport is reversed in response to depolarization. This outward transport is induced by small increases in the extracellular K^+ concentration and by high-frequency neuronal firing, and it can exert a significant inhibitory effect on surrounding neurons through activation of $GABA_A$ receptor-mediated tonic inhibition.[336] The outward transport of GABA is enhanced by vigabatrin and gabapentin, and may provide a significant contribution to the anticonvulsant activity of these drugs.[337] Other AEDs may also alter the function of GABA transporters. For example, therapeutic concentrations of valproate can produce a small enhancement of GABA-induced transporter currents in hGAT-1, mGAT-1 and mGAT-4 expression systems, suggesting that GABA transport may be promoted.[338]

Plasma membrane glutamate transporters

Five glutamate transporters (EAAT1–5) are expressed in neuronal and astrocytic plasma membranes[339–341] (TABLE 9). They mediate a high-affinity sodium-dependent uptake of glutamate and aspartate. Glutamate is cotransported with one H^+ and three Na^+, and one K^+ is counter-transported. Although their primary function appears to be removal of glutamate from the synaptic cleft, the transporters influence glutamatergic transmission by controlling the extracellular concentration of glutamate available to activate metabotropic receptors at presynaptic, perisynaptic, and nonsynaptic sites, and also nonsynaptic NMDA receptors.[342] Glutamate is the precursor for GABA synthesis, and there is evidence that EAAC1 expressed in GABAergic terminals contributes to the glutamate uptake required to maintain GABA levels.[343]

GLAST (EAAT1) is predominantly astroglial during development; in the adult it is expressed in Bergmann glia. GLT-1 (EAAT2) is universally expressed in astrocytes and accounts for 90% of the glutamate uptake in the adult brain. GLT-1b (EAAT2b) is a splice variant with 11 fewer C-terminal amino acids found in neurons and astrocytes. Various glutamate transporter-associated proteins (GTRAP 3-18, GTRAP 41, GTRAP 48) interact with the C-terminal of the transporter molecules and influence their function and trafficking.

The glutamate transporters also mediate a Cl^- conductance that is not linked to the Na^+ and glutamate translocation. This anion current is most marked in EAAT4 and EAAT5. These two neuronal glutamate transporters

TABLE 9. *Glutamate Transporters: Cellular Localization, Genetic Syndromes Associated With Epilepsy and Pharmacology*

Transporter	Cellular Localization	Astrocytes, Bergmann glia	Drug Effects
GLAST/EAAT1 (*SLC1A3*)	Astrocytes, Bergmann glia	Knockout mice → enhanced PTZ susceptibility; mutation in human → EA6	
GLT-1/EAAT2	Astrocytes (GLT1b/EAAT2b splice variant in astrocytes and neurons)	Knockout mice → seizures	Ceftriaxone (enhances expression); *threo*-3-methylglutamate, dihydrokainate (*inhibitor*)
EAAC1, EAAT3	Neurons (postsynaptic)		
EAAT4	Purkinje cells		*threo*-3-Methylglutamate (*inhibitor*)
EAAT5	Retina		

EA6 = episodic ataxia/hemiplegic migraine/seizures (mutation in *SLC1A3*).

clearly have a very different function from the glial transporters. Presynaptically, they interact with metabotropic receptors and influence the release of glutamate and GABA. Postsynaptically, they are perisynaptic and interact closely with mGluRs.[340]

There is only a single case report of a mutation in a glutamate transporter associated with seizures.[344] This was in a 10-year-old boy with episodic ataxia, seizures, migraine, and alternating hemiplegia (EA6); a C→G transversion was found in the *SLC1A3* gene. The mutant EAAT1 transporter showed impaired glutamate uptake, and when coexpressed with wild-type EAAT1, decreased its activity.

Various alterations in glutamate transporters in mice have been associated with seizures; for example, mice lacking GLT-1 exhibit seizures and hippocampal pathology and experience early death.[345] Mice lacking GLAST show enhanced seizure susceptibility with PTZ, but interpreting the mechanism is complicated by marked compensatory changes in the expression not only of the other glutamate transporters but also of various types of glutamate ionotropic receptors.[346] EAAC1 null mice show dicarboxylic aminoaciduria (EAAC1 has a functional role in the kidney), but no seizures. Knockdown of EAAC1 by antisense probes in adult rats produces seizures apparently by impairing GABA synthesis.[340] EAAT4 null mice show no abnormal phenotype.

Studies of glutamate transporter mRNA or protein levels in tissue removed from patients with temporal lobe epilepsy have not shown significant reductions in glutamate transporter expression; rather some increases in EAAT1 and EAAT3 have been reported.[347–349]

Many glutamate analogs such as β-hydroxyaspartate, dihydrokainate, and L-*trans*-2,4-pyrrolidine dicarboxylate act as selective inhibitors of glutamate transporters and have been used to study their physiological roles.[350] A potent nontransportable EAAT1–4 inhibitor, TFB-BOA, produces epileptiform discharges in hippocampal slices and severe convulsions in mice.[351,352]

Because excessive extracellular glutamate can lead to seizures, it is apparent that tight regulation of extracellular glutamate is critical to seizure control.[353] Glutamate transporters are therefore potential AED targets. Clearly, what is required is a compound that either directly activates EAAT2 (or EAAT1 or EAAT3) or enhances its expression at the cell surface. Both approaches appear feasible.[354] Small molecules that activate glutamate uptake *in vitro* have been identified, including the neuroprotective agent (*R*)(−)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153)[355] and a component of a spider venom.[356] Moreover, EAAT2 expression in organotypic spinal cord slice cultures can be enhanced sixfold by β-lactam antibiotics such as penicillin and ceftriaxone.[357] Whether either of these approaches will provide seizure protection remains to be determined.

Vesicular glutamate transporters

There are three vesicular glutamate transporters. The first of these to be identified, VGLUT1, was assigned as a vesicular glutamate transporter after having first been identified as an inorganic phosphate carrier.[358,359] Subsequently, VGLUT2 and VGLUT3 were cloned and characterized and shown to have distinctive distributions in the brain. These transporters have a much lower affinity for glutamate than EAAT1–5 and, unlike the plasma membrane transporters, they do not transport D- and L-aspartate. They are activated by low concentrations of Cl^-.

VGLUT1 is prominent in glutamatergic terminals in cortex, cerebellum, and hippocampus; VGLUT2 is present in glutamatergic terminals in midbrain and hindbrain; and VGLUT3 appears in nonglutamatergic neurons during development, being colocalized in terminals with VGAT, with VAC, and with VMAT (suggesting a

special role in providing the corelease of transmitters).[360] There is evidence that VGLUT1 may be upregulated in models of epileptogenesis.[361] The dye compounds Evans Blue and Rose Bengal powerfully inhibit glutamate uptake by VGLUT1–3.[362] No known anticonvulsant substances modulate vesicular neurotransmitter transporters, but they do represent attractive potential targets.

PRESYNAPTIC PROTEINS INFLUENCING SYNAPTIC FUNCTION

Synaptic vesicle proteins

Several proteins are known to influence synaptic vesicle formation and function. These include the synapsin proteins (synapsin I–III) that coat the vesicles and regulate transmitter release.[363] Mice deficient in synapsin I exhibit enhanced seizure-like electrographic and behavioral responses with amygdala stimulation.[364] Synaptic function in these animals is altered in a variety of ways. In particular, there is a loss of immediately releasable GABA, which could account for the hyperexcitability.[365,366] The synapsins are regulated by phosphorylation, which might provide a mechanism for pharmacological manipulation.

SV2A is an abundant protein component of synaptic vesicles that is structurally similar to 12-transmembrane domain transporters, although a transporter activity has not yet been identified. It is a member of a family of three related synaptic vesicle proteins (SV2A, SV2B, and SV2C). The functions of SV2 proteins are not fully understood. It has been proposed that the proteins interact with synaptotagmin 1[367] to cause an enhancement of low-frequency neurotransmission by priming docked vesicles.[368] Specificially, SV2 is believed to prime vesicles in quiescent neurons so that low-frequency neurotransmission is faithfully conveyed.[369]

The AED levetiracetam and its analogs brivaracetam and seletracetam bind specifically to SV2A and not to the other SV2 proteins.[370–373] Structure–activity studies indicate that this interaction accounts for the anticonvulsant activity of the drugs.[10] SV2A is not essential for synaptic transmission, but knockout of the protein in mice leads to severe seizures (in constrast to SV2B knockout mice, which do not show seizures).[367,368] The way in which binding of levetiracetam and related compounds to SV2A influences seizure susceptibility is not understood. However, the identification of SV2A as an AED target demonstrates the potential of targeting proteins other than ion channels for AED development.

Synaptic anchoring proteins

Many proteins are involved in the anchoring and trafficking of ion channels, receptors, and transporter molecules. A large group of these proteins are found in postsynaptic densities, including spectrin, actin, calcineurin, contactin, PSD-95, SAP-90, gephyrin, and Homer 1A. The expression of many of these molecules changes during the course of kindling or status epilepticus-induced epileptogenesis. Such changes may contribute to or oppose epileptogenesis. For example, overexpression of Homer 1A during kindling may modulate glutamatergic transmission and oppose seizure activity and epileptogenesis.[374] In addition to Homer 1A, the many other proteins that participate in the trafficking of receptor subunits are potential targets for epilepsy therapy, because trafficking undoubtedly plays a part in epileptogenesis.

ENZYMES

Two enzymes, GABA-transaminase and carbonic anhydrase, are recognized AED targets. Enzymes involved in the regulation of receptor phosphorylation are other potential AED targets.

GABA-transaminase

Synaptically released GABA is transported into astrocytes and nerve terminals, where it is either taken up into synaptic vesicles or further metabolized by two mitochondrial enzymes, GABA-transaminase (4-aminobutyrate-2-oxoglutarate aminotransferase) and succinic semialdehyde dehydrogenase, to yield succinate, a tricarboxylic acid cycle intermediate. After it was shown that inhibitors of GABA-transaminase raise brain GABA content and inhibit seizures in rodents,[375] two GABA analogs, γ-acetylenic GABA and γ-vinyl GABA (vigabatrin), were identified as irreversible inhibitors of GABA-transaminase with prolonged anticonvulsant activity in rodent and primate models of epilepsy.[7,376,377] They initially bind reversibly to the cofactor pyridoxal-5-phosphate and then bind covalently to the active site of the enzyme.[378] Vigabatrin is a racemic mixture; the *S*(+)-enantiomer possesses the anticonvulsant activity.[379] In mice, an anticonvulsant dose of vigabatrin increases brain GABA content five- to sixfold.[7] In humans, vigabatrin increases cerebrospinal fluid GABA concentration by 180%[380] and occipital cortical GABA content threefold.[381,382]

The hypothesis that the anticonvulsant action of vigabatrin is a consequence of enhanced GABAergic inhibition resulting from an augmented releasable pool of neurotransmitter has been abandoned, following the observation that IPSCs are diminished in frequency and amplitude in cultured hippocampal neurons exposed to vigabatrin.[337,383] What is clear from *in vitro* and *in vivo* studies is that the extracellular concentration of GABA is increased, leading to some desensitization of synaptic $GABA_A$ receptors, but also to an increase in tonic Cl^- conductance due to activation of nonsynaptic $GABA_A$ receptors.[337] The increase in extracellular GABA is not

dependent on vesicular release, but rather on reverse transport of GABA by GAT-1 or GAT-3.

The clinical use of vigabatrin has been severely restricted by the discovery that it causes ganglion cell damage, resulting in a high incidence of visual field defects.[384] It is not clear whether the retinal pathology is a direct consequence of GABA-transaminase inhibition or is related to some other effect of vigabatrin, such as glutamic acid decarboxylase inhibition. Until it is shown that chronic inhibition of GABA-transaminase sufficient to suppress seizures can be achieved without risk of retinal damage, the utility of GABA-transaminase as an AED target will remain uncertain.

Carbonic anhydrase

Carbonic anhydrase (carbonate dehydratase) is an enzyme found in diverse isoforms in most tissues, including blood and brain. By controlling the rate of hydration of CO_2 and dehydration of bicarbonate (HCO_3^-), it modulates intracellular and extracellular pH. Specifically, carbonic anhydrase inhibition causes extracellular acidification because of interference with H^+ buffering; there may also be parallel intracellular acidification.

Neuronal excitability is influenced by pH in many different ways. Probably most important in epilepsy is modulation of the functional consequences of $GABA_A$ receptor activation, which occurs through intraneuronal carbonic anhydrase VII.[385] Although $GABA_A$ receptor currents are carried mainly by Cl^-, HCO_3^- normally carries an opposing outward (depolarizing) current that is one fifth the size of the Cl^- current. During intense $GABA_A$ receptor activation, intracellular accumulation of Cl^- ions and the CO_2-mediated redistribution of HCO_3^- ions can lead to a situation in which outward HCO_3^- current exceeds inward Cl^- current, resulting in depolarizing GABA response.[386] This effect, which is dependent on the intraneuronal generation of HCO_3^- by carbonic anhydrase VII, probably plays a significant role in hippocampal epileptiform activity.[387] Carbonic anhydrase in astrocytes also can influence neuronal excitability by regulating extracellular pH. Decreasing pH tends to depress epileptiform burst firing, through a variety of actions,[388] including effects on NMDA receptors, which are inhibited by extracellular H^+.[389]

Acetazolamide and various related sulfamates were shown in the 1950s to inhibit carbonic anhydrase and to be anticonvulsant in rodent seizure models (more effective against MES than against PTZ seizures) and in humans against generalized and focal motor seizures and absence epilepsy.[390,391] Tolerance develops relatively rapidly, however, largely due to enhanced carbonic anhydrase activity. Two more recently introduced AEDs, zonisamide and topiramate, also inhibit carbonic anhydrase, which may contribute to their side effects, including kidney stones and perhaps also oligohydrosis and hyperthermia.[392,393]

The action of topiramate on carbonic anhydrase has been assumed not to contribute to its clinical efficacy, because cross-tolerance to the anticonvulsant activity of topiramate does not occur with acetazolamide in mice.[394] However, a recent study finds closely comparable potency for acetazolamide and the two AEDs against cytosolic carbonic anydrase isozyme II and mitochondrial carbonic anhydrase isozyme V.[395] Carbonic anhydrase is a zinc-containing enzyme that is inhibited by interaction of sulfonamide or sulfamate compounds with the Zn(II) ion and specific residues of the protein. X-ray crystallography of human carbonic anhydrase II with zonisamide demonstrates a classical interaction with the protein that is characteristic of sulfonamides, as is zonisamide. Another recent study suggests, however, that although both topiramate and acetazolamide can induce a slow outward K^+ current in hippocampal neurons (yet another possible mechanism of topiramate[396]), neither drug exerts this action through effects on carbonic anhydrase.[397]

Because of the problem of tolerance, carbonic anhydrase has not been considered a promising target for new AED development. This situation may change if the propensity for tolerance can be overcome, perhaps by selective targeting of isoforms with intracellular–extracellular or neuronal–astrocytic specificity.

Protein kinases and phosphatases

Activation of either ionotropic or metabotropic receptors has immediate effects on ionic conductances and delayed effects involving second messenger systems. These longer-term effects may be triggered by changes in intracellular Ca^{2+} that influence the activity of a large number of enzymes (such as Ca^{2+}/calmodulin-dependent protein kinase) or through changes in second messengers, such as cAMP and cGMP, that affect protein kinases.

Phosphorylation sites are present on most voltage-gated ion channels, receptors, and membrane transporter molecules, and the phosphorylation state of these sites may modulate the activity of the effectors. There is no firm evidence that altered phosphorylation of ion channels or other excitability molecules is responsible for epileptogenesis. There are, however, numerous reports of functional changes in voltage-gated ion channels, GABA and glutamate receptors, and transporters in brain tissue from rodents with kindled seizures and humans with intractable temporal lobe epilepsy.[398–400] Some of these probably depend on altered gene expression, but many could result from altered phosphorylation. Mutations involving kinase or phosphatase systems would have such widespread effects on development and cellular function that they are unlikely to be found associated with idiopathic epilepsy. Similarly, pharmacological agents that

influence these enzymes might not be expected to have sufficiently specific actions to be useful anticonvulsants. As noted previously, however, topiramate may act indirectly on ion channels by effects on phosphorylation.[29]

GAP JUNCTIONS (CONNEXINS)

Evidence for a role of gap junctions in epilepsy has come to the fore in the last decade.[27,401,402] Electrotonic coupling between vertebrate neurons was described in the 1960s and 1970s, prior to the description from electron micrographs of gap junctions between neurons. Structurally these comprise two hemichannels (connexons), each made up of six connexin proteins surrounding a central pore that permits the passage of ions, second messengers, and other small molecules between cells. Gap junctions can be between neurons or between astrocytes (homocellular) or between astrocytes and neurons (heterocellular). Hemichannels may also permit the release of compounds such as glutamate and ATP from glia into the extracellular fluid.

About 20 connexins have been identified in mammals. Connexins (Cx) 26, 29, 30, 32, 36, 37, 40, 43, 45, 46, and 47 are expressed in the brain, with marked variation according to developmental stage and cell type.[402] In astrocytes, where a key function for gap junctions is calcium wave signaling, Cx43 is the major connexin.[403] In neurons, Cx36 and Cx45 play an important role and provide electrical coupling between neurons in cortex and hippocampus.[404] In oligodendrocytes, Cx32 and Cx47 provide nutrients to the myelin sheaths.[405]

Connexin knockout studies in mice present a very mixed picture of developmental defects and some changes in oscillatory potentials in slice preparations,[406,407] but are difficult to interpret because of possible compensatory changes in other connexins. Recent studies indicate that knockout of the major neuronal gap junction protein Cx36 promotes epileptic hyperexcitability, suggesting that specific inhibitors would not be useful as AEDs. Other connexins may be relevant, however. Changes in the expression of connexins may play a role in epileptogenesis.[408,409] Moreover, there is strong evidence that gap junctions play a role in the fast oscillations that precede the onset of seizure discharges in the hippocampus.[27,410]

In brain slice preparations, drugs that block gap junction function (such as halothane, octanol, or carbenoxolone) suppress epileptiform discharges, including those induced by zero Ca^{2+}.[411–413] Opening channels with trimethylamine or alkalinization augments discharges.[414] *In vivo* studies with gap junction blockers and openers have recently been performed in genetic and acquired rodent models of absence seizures.[415–417] These studies have established that systemically administered carbenoxolone can diminish spike-and-wave discharges and indicate that gap junctions play a role in the spread of seizure activity in the thalamus and the cortex. Moreover, the data identify connexins as plausible molecular targets for AED development.

The selective expression of the isoforms in specific neurons and astrocytes provides the opportunity for precise molecular targeting. Recent evidence implicating astrocytes in the generation of epileptic activity suggest that selective targeting of astrocytic gap junctions, such as Cx43, may be a fruitful strategy.[418] The novel benzoylamino benzopyran tonabersat [SB-220453; an analog of the anticonvulsant carabersat (SB-204269)[419]], which is currently in clinical development for migraine, is believed to block gap junctions.[420]

DRUG DEVELOPMENT FOR SPECIFIC EPILEPSY SYNDROMES

A critical aim of AED research is to provide a rational basis upon which physicians can select the best drug for each patient based on that patient's specific seizure types and epilepsy syndrome. Moreover, in light of the increased understanding of epilepsy mechanisms, it should be possible to develop improved AEDs that are specifically tailored to an individual patient's underlying pathophysiology. Needless to say, neither of these goals has as yet been approached.

This review has suggested numerous potential targets on which to base the development of the next generation of more specific AEDs. In cases where alterations in ion channels or other molecules believed to regulate synaptic transmission or neuronal excitability are known to underlie the epilepsy sydrome, it is logical to design a drug to counteract the effect of the functional change, whether it is a mutation or change in expression of voltage-gated Na^+, Ca^{2+}, or K^+ channels, $GABA_A$ receptor subunits, or h-channels. In other situations, it may be rational to specifically target a normal ion channel or other excitability molecule to balance the effect of a defective molecule. Although AEDs have not yet been developed based on such rational design principles, we give two examples where they could be applied.

Molecular targets for the treatment of absence seizures

The neural pathways involved in the spike-and-wave discharges of absence seizures have been well defined in rodent models of absence seizures,[421–423] and a great deal is known about the pharmacological properties of the receptors and ion channels involved in the discharges[424] (TABLE 10). The current consensus is that such discharges are initiated by spikes in a small cortical region (the perioral area in the somatosensory cortex) that provides a powerful glutamatergic input to GABAergic neurons in the thalamic reticular nucleus

TABLE 10. *Molecular Targets of Known or Potential Relevance to Absence Seizures*

Target	Location	Alteration in Absence Epilepsy Models	AEDs Effective in Absence Epilepsy and Absence Models*
T-type voltage-gated Ca^{2+} channels	TRN and thalamocortical relay neurons		Ethosuximide, trimethadione, zonisamide
P/Q, N-type voltage-gated Ca^{2+} channels			Lamotrigine, ? levetiracetam (N-type)
$Na_v1.1$, $Na_v1.6$		Upregulated in cortical focus in WAG/Rij rats	*Carbamazepine, phenytoin (exacerbate)*
HCN2 (also HCN1)		Knockout HCN2 → absence-like seizures; HCN1 expression reduced in cortex of WAG/Rij rats	Lamotrigine increases I_h
$GABA_A$ ($\alpha3\beta3\gamma2$)	TRN		Clonazepam; *vigabatrin, tiagabine (exacerbate)*
$GABA_B$		Upregulated in thalamus and cortex in GAERS model	CGP 35348, CGP 36742; *baclofen (induces spike-and-wave discharges)*
NMDA receptor	Thalamus, neocortex	Potentiated in GAERs	NMDA antagonists inhibit; *NMDA (i.c.v.) increases*
AMPA receptor			AMPA antagonists focally injected into the perioral region of the primary somatosensory cortex (S1po) of WAG/Rij rats block absence-like seizures; systemic AMPA receptor antagonist talampanel poorly effective
mGluR1 (group I)	TRN, thalmaic relay neurons, cortex	Function altered in WAG/Rij	
mGluR2/3 (group II)			LY341495 (group II mGluR antagonist) reduces spike-and-wave dicharges in WAGg/Ri rats
Connexins	TRN, thalamic relay neuron, cortex		Carbenoxolone (focal injections into TRN or nucleus ventralis posterolateralis in WAG/Rij rats or i.c.v. in *lh/lh* mice)

*Italic type indicates exacerbation of absence seizures.
i.c.v. = intracerebroventricular; TRN = thalamic reticular nucleus.

(TRN) and glutamatergic thalamic relay nuclei. The GABAergic input from the TRN hyperpolarizes relay neurons, converting their background fast oscillations to slow oscillation (2–3 Hz in humans, 5–7 Hz in rodents) that are paced by the properties of the T-type Ca^{2+} currents in the TRN and relay nuclei. Glutamatergic outputs from the thalamus to deep cortical neurons and reciprocal glutamatergic inputs to the thalamus are essential to the maintenance of the spike-and-wave discharge.

Since the original report that ethosuximide decreases T-type Ca^{2+} currents in thalamic neurons,[72] many have doubted that this effect explains the antiabsence action of ethosuximide, partly because it is relatively weak and is not seen in many cellular preparations.[73,154] Nevertheless, the effect on T-type currents is undoubtedly real, because it has been demonstrated in expressed human receptors[74]; it is probably large enough to block slow oscillatory firing. Microinfusion of ethosuximide into the thalamus is, however, less effective at blocking spike-and-wave discharges than infusion into the somatosensory cortex, suggesting that molecular targets relating to the initiation of spikes in the cortex (e.g., Na^+ channels, HCN channels) may be more important than thalamic T-type Ca^{2+} channels.

There is a selective upregulation of mRNA and protein of $Na_v1.1$ and $Na_v1.6$ in layer II–IV neurons in the facial somatosensory cortex in WAG/Rij rats compared with Wistar rats,[425] supporting the idea that altered Na^+ channel function may contribute to the onset of absence seizures. There is also evidence in this model of a reduction in HCN1 protein and I_h currents in pyramidal neurons in the somatosensory cortex.[164] It has been noted previously that lamotrigine enhances I_h, so that it could theoretically reverse this reduction.[170] Lamotrigine is the only classical Na^+ channel-blocking AED with efficacy in the treatment of absence epilepsy; its unique activity could be due to specific biophysical differences in its action on Na^+ channels or to additional actions, such as the effect on I_h.

TABLE 11. *Molecular Targets of Known or Potential Relevance to Limbic Partial Seizures*

Target	Function	AED/Potential AED
$GABA_A$ receptors	Phasic and tonic inhibition	Barbiturates, benzodiazepines, neurosteroids
GABA-transaminase	Tonic inhibition	Vigabatrin
GAT-1	Tonic inhibition	Tiagabine
M-type K^+ channels	Set resting potential and limit excitation near spike threshold	Retigabine
A-type K^+ channels	Regulation of neurotransmitter release (presynaptic); limit high-frequency spike firing	
HCN1	Pacemaking activity	Lamotrigine
Connexins	Electrical synchronization, fast oscillations	Carbenoxolone
G-protein-coupled receptors and associated K_{ir}/voltage-gated Ca^{2+} channels	Presynaptic regulation of neurotransmitter release	Ligands acting on receptors for monoamines, neuropeptides (neuropeptide Y, galanin, somatostatin)

Molecular targets for the treatment of limbic seizures

Although many currently available AEDs have some efficacy in the treatment of temporal lobe epilepsy, a substantial proportion of patients do not obtain complete seizure relief with any of them. If there are AEDs highly selective for limbic temporal lobe seizures, they are likely to have remained undiscovered up to now because they are not detected by the AED screening models in common use. Nevertheless, most currently available AEDs, including those that act via Na^+ channels and GABA systems, do have activity in the treatment of partial seizures, including those in temporal lobe epilepsy.

GABAergic inhibition is highly important in limbic seizures[426] and agents acting via $GABA_A$ receptors, GABA-transaminase and GAT-1 are effective in kindled seizures and complex partial epilepsies (TABLE 11). Thus, GABA systems continue to be relevant targets for drugs to treat temporal lobe seizures. Electrophysiological studies in temporal lobe slice preparations attribute important roles to K^+ channels in altered excitability. The M-type current (mediated by $K_v7.2$–7.5) is proven as a target by the effect of retigabine and other channel openers in amygdala-kindled seizures. Presynaptic A-type K^+ currents are also particularly significant,[427] because genetic defects in $K_v1.1$ in mice and humans are associated with partial or limbic seizures.[35,114–116] The channels responsible for the A-type current would therefore appear to be promising targets. Other channels that have been discussed in this review that would seem to be appropriate targets include HCN channels (important in controlling hippocampal excitability[162,428]), connexins, and G-protein-coupled receptors and their associated K_{ir} channels.

CONCLUSIONS

A remarkable array of molecular targets is available on which to base the rational development of new and potentially improved AEDs. Because epilepsy is fundamentally a disorder of neuronal excitability, we have focused in this review on ion channels—the molecular mediators of excitability—and on other proteins that influence the activity of these ion channels, such as auxiliary subunits, and also neurotransmitter transporters and enzymes that affect the disposition of neurotransmitters.

In recent years, it has become apparent that these excitability molecules are the predominant cellular components affected in genetic epilepsy syndromes, reinforcing their importance in epilepsy and as targets for AEDs. Thus, voltage-gated and ligand-gated ion channels are the most important class of targets for currently marketed AEDs, and they continue to hold promise as targets for the development of future AEDs. Improved AEDs may result from more precise targeting of specific subunits to obtain greater specificity, and also from considering as potential targets the many associated proteins that modify the functional properties of these channels or alter their trafficking or local expression. There is still the prospect that better AEDs may be identified that target the α subunits of voltage-gated Na^+ channels or the α or auxiliary subunits of presynaptic voltage-gated Ca^{2+} channels.

In addition, the broad range of K^+ channels offer many unexploited molecular targets, particularly the channels generating the A-type and M-type currents. The potential for compounds that potentiate M-type K^+ currents is evident from the recognition that the powerful anticonvulsant activity of retigabine results from its action on the $K_v7.2/K_v7.3$ channels that underlie the M-current. Other members of the voltage-gated ion channel superfamily, including inwardly rectifying, Ca^{2+}-acti-

vated K^+ channels, and HCN channels are intriguing potential targets, but have not yet been validated.

$GABA_A$ receptors, responsible for phasic and tonic inhibition, are the principal ligand-gated ion channel targets for AEDs. These receptors continue to represent attractive targets for AED development. Understanding how the various subunits determine the functional roles of $GABA_A$ receptors along with continued developments in the pharmacology of these receptors should permit the design of improved AEDs acting via these targets.

Ionotropic glutamate receptors, especially NMDA and AMPA receptors, have been identified as relevant targets in animal models. Reducing excess excitation by blockade of postsynaptic NMDA receptors may not be a viable clinical approach, however; there is evidence that AMPA receptor antagonists may have greater clinical potential, especially for the treatment of status epilepticus.

The anticonvulsant activity of Na^+ channel blocking AEDs likely resides in their ability to reduce the synaptic release of glutamate. Therefore, other strategies to modulate glutamate release (such as the targeting of presynaptic metabotropic receptors, presynaptic voltage-gated Na^+, K^+ or Ca^{2+} channels, or the wide range of proteins involved in the accumulation of neurotransmitters in synaptic vesicles and their release into the synaptic cleft) are intriguing for AED development. Recent evidence indicating that gap junctions between neurons and astrocytes may play a role in the pathophysiology of seizure discharges suggests that connexins may also be promising targets.

Attempts have been made to rationally design AEDs for nearly four decades, with only limited success. The current vastly improved understanding of the molecular targets, coupled with advances in the pathophysiology of epilepsy, which include a succession of breakthroughs in genetics that have defined the fundamental molecular defects of many epilepsy syndromes, provides a platform for greater success in the future.

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