Mechanisms of Action of Currently Used Antiseizure Drugs

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Dedication

The authors dedicate this article to the memory of Professor Brian S. Meldrum, a colleague, collaborator, dear friend, and a giant in epilepsy and antiseizure drug research.

Abstract

Antiseizure drugs (ASDs) prevent the occurrence of seizures; there is no evidence that they have disease-modifying properties. In the more than 160 years that orally administered ASDs have been available for epilepsy therapy, most agents entering clinical practice were either discovered serendipitously or with the use of animal seizure models. The ASDs originating from these approaches act on brain excitability mechanisms to interfere with the generation and spread of epileptic hyperexcitability, but they do not address the specific defects that are pathogenic in the epilepsies for which they are prescribed, which in most cases are not well understood. There are four broad classes of such ASD mechanisms: (1) modulation of voltage-gated sodium channels (e.g. phenytoin, carbamazepine, lamotrigine), voltage-gated calcium channels (e.g. ethosuximide), and voltage-gated potassium channels [e.g. retigabine (ezogabine)]; (2) enhancement of GABA-mediated inhibitory neurotransmission (e.g. benzodiazepines, tiagabine, vigabatrin); (3) attenuation of glutamate-mediated excitatory neurotransmission (e.g. perampanel); and (4) modulation of neurotransmitter release via a presynaptic action (e.g. levetiracetam, brivaracetam, gabapentin, pregabalin). In the past two decades there has been great progress in identifying the pathophysiological mechanisms of many genetic epilepsies. Given this new understanding, attempts are being made to engineer specific small molecule, antisense and gene therapies that functionally reverse or structurally correct pathogenic defects in epilepsy syndromes. In the near future, these new therapies will begin a paradigm shift in the treatment of some rare genetic epilepsy syndromes, but targeted therapies will remain elusive for the vast majority of epilepsies until their causes are identified.

1. Introduction

Drugs used in the treatment of epilepsy are taken chronically to prevent the occurrence of seizures. In broad terms, they influence fundamental brain excitability mechanisms to suppress abnormal hyperexcitability and hypersynchronous activity in brain circuits. Antiseizure drugs (ASDs) do not necessarily have specific actions related to the underlying pathogenic mechanisms in epilepsy, which in most cases are not understood. In the past two decades, the molecular defects in many genetic epilepsies have been characterized and there is an intense interest in the development of disease-specific targeted therapies. Early examples of this effort include everolimus, an inhibitor of mTOR signalling used in tuberous sclerosis, and cerliponase alfa, used in the treatment of the CLN2 form of Batten disease. Focus is also being directed toward antisense approaches and gene therapies with viral vectors, but small molecules that interact with diseased proteins, such as ion channels with gain or loss of function mutations, are also being investigated. A theoretical advantage of such mechanism-based therapies is that they have the potential to not only reduce the occurrence of seizures but also to prevent or reverse comorbidities, such as neurological impairments that are common in such syndromes.

Early ASDs were identified serendipitously when they were administered to people with epilepsy (bromide was introduced in 1857 and phenobarbital in 1912). Testing in animal models led to the discovery of phenytoin in 1936 and has been notably successful ever since, with more than 30 distinct molecular entities entering clinical practice as a result of this approach. Several other ASDs were rationally developed based on mechanism (e.g., tiagabine, vigabatrin, perampanel) and others represent minor chemical modifications of existing drugs (e.g., fosphenytoin, various benzodiazepine forms, oxcarbazepine, eslicarbazepine acetate, brivaracetam). None of these drugs have been demonstrated to have disease modifying properties; they simply treat symptoms (reduce the occurrence of seizures). As such, the term “antiepileptic drug” has fallen out of favour, having been replaced by the designation “antiseizure drug” as used in this article.

Recurrent seizure activity is the manifestation of intermittent and excessive hyperexcitability in localized cortical or limbic circuits in focal-onset epilepsies or more diffuse networks in generalized epilepsies. Four broad classes of ASD mechanism have recently been recognised: (1) modulation of voltage-gated ion channels; (2) enhancement of GABA-mediated inhibitory neurotransmission; (3) attenuation of glutamate-mediated excitatory neurotransmission; and (4) modulation of neurotransmitter release via a presynaptic action (Table 1; Rogawski and Cavazos, 2020). A fifth class represents the mechanism-targeted agents, exemplified by everolimus. There is obvious overlap in these mechanistic classes, particularly for those drugs in class 1 and class 4, where alteration in ionic currents that underlie neuronal excitability has downstream effects on neurotransmitter release at synapses, with glutamate release seemingly diminished to a greater extent than that of GABA (Prakriya and Mennerick, 2000). Some ASDs are likely to prevent seizures via actions on multiple cellular targets; the combination of effects may contribute to efficacy while limiting adverse effects mediated by any individual mechanism. The mechanism of action of several ASDs, including the important agents valproate and levetiracetam, remain elusive even after several decades of clinical use (Löscher, 2002). Nevertheless, the primary mechanisms of action of the majority of currently used drugs is now reasonably well delineated; these are discussed in detail below.

2. Modulation of voltage-gated ion channels

2.1 Blockade of voltage-gated sodium channels

Voltage-gated sodium channels are responsible for depolarisation of the nerve cell membrane during the upstroke of action potentials and are critical to the propagation of action potentials across the surface of neuronal cells. They are expressed throughout the neuronal membrane, on dendrites, soma, axons, and nerve terminals (Catterall, 2017). Density of expression is highest in the axon initial segment where action potentials are generated. Voltage-gated sodium channels comprise a single 260 kDa α-subunit protein that is arranged into four homologous domains (I-IV) each consisting of six transmembrane segments (S1-S6) (Catterall and Swanson, 2015). The S4 segment of each domain has a high proportion of charged amino acids and acts as the voltage sensor, while the S5 and S6 segments contain hydrophobic residues that line the intrinsic channel pore. In the central nervous system, the α-subunit is commonly associated with two accessory β-subunit proteins (β-1 and β-2) that can influence channel kinetics and the voltage-dependence of gating, but which are not essential for the sodium conducting properties of the channel (Hull and Isom, 2018).

Of the nine mammalian sodium channel α-subunit genes, five are expressed in the brain: *SCN1A*, *SCN2A*, *SCN3A*, *SCN5A* and *SCN8A*, encoding the channels $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.5$ and $Na_V1.6$, respectively (Catterall, 2017). $Na_V1.3$ expression is largely restricted to the early stages of development; $Na_V1.5$, the main cardiac sodium-channel isoform, is also expressed throughout the brain but its role is not well understood, and $Na_V1.1$ is the major voltage-gated sodium channel in inhibitory interneurons (Whitaker et al., 2000; Wang et al., 2017). In contrast, the $Na_V1.2$ and $Na_V1.6$ channels are expressed in the axon initial segment of principal excitatory neurons, the former predominating in the immature brain and the latter becoming increasingly prevalent during development (Whitaker et al., 2000). The $Na_V1.6$ channel also carries a significant proportion of the persistent sodium current that has been implicated in burst firing and ictogenesis (Stafstrom, 2007). Under normal physiological conditions, depolarisation of the neuronal membrane leads to a transient inward sodium current which rapidly inactivates. However, a small proportion of sodium channels appear to undergo rare, late openings in response to depolarisation and give rise to a sodium current that fails to inactivate, and is thereby termed “persistent” (Crill, 1996). The existence of this non-inactivating sodium current is relevant to the pharmacology of some ASDs (see below).

Voltage-gated sodium channels exist in one of three basic conformational states; (i) at hyperpolarised potentials the channel is typically found in a resting, closed state, (ii) when depolarised the channel transitions to an open state that is permeable to sodium ions, and (iii) following depolarisation the channel enters a closed, non-conducting inactivated state (Catterall, 1992; Catterall, 2017). During a single round of depolarisation, channels cycle through these states in turn – resting to open, open to inactivated, inactivated to resting – and the ability of individual channels to contribute to subsequent membrane depolarisations is governed by the rate at which they revert from the inactivated to resting state. Two distinct inactivation states of the voltage-gated sodium channel are now recognised; a fast inactivated state that is conferred by a "hinged lid" formed from the intracellular loop between domains III and IV that transiently (milliseconds duration) blocks the ion pore following short depolarisations, and a slow inactivated state that is conferred by a longer lasting (seconds duration) conformational change in the α-subunit protein which is observed following prolonged depolarisations (Silva, 2014). Modification of slow inactivation has been proposed as a mechanism for certain ASDs, but recent work calls this notion into question (Jo and Bean, 2017).

Blockade of voltage-gated sodium channels is the most common mechanism of action among currently available ASDs. The established agents phenytoin and carbamazepine are archetypal sodium channel blockers, an effect they share with the newer drugs lamotrigine, oxcarbazepine, lacosamide, and S-licarbazepine, which is the active metabolite of the prodrug eslicarbazepine acetate (Ragsdale et al., 1991; Mantegazza et al., 2010). Rufinamide also acts at least in part via voltage-gated sodium channels, possibly with modest preferential activity on $Na_V1.1$ and $Na_V1.6$ (Gilchrist et al., 2014), but other mechanisms are likely given its distinctive clinical profile. Topiramate, felbamate and zonisamide have also been reported to block sodium channels, as one of several possible mechanisms. Despite their structural dissimilarities, there is believed to be a common binding site for ASDs on the α-subunit of the voltage-gated sodium channel, which is found on the inner pore region of domain IV, transmembrane segment S6 (Kuo, 1998). Differences in efficacy and adverse effects of selective sodium channel blocking ASDs are explained by differences in their rates of binding (i.e., their affinities) and also in their mechanisms of unbinding or dissociation (Kuo et al., 1997). Much of the work in this area has focused on differences between phenytoin and carbamazepine, with the former appearing to possess a slower onset of binding and a similarly slow dissociation that is driven by deactivation of the channel (Kuo and Bean, 1994). As such, phenytoin appears to have a more pronounced and longer lasting effect than carbamazepine on high frequency action potential firing.

Another common feature of ASDs with sodium channel blocking properties is their preferential affinity for the channel protein when it exists in the inactivated state (Schwarz and Grigat, 1989). Binding slows the conformational recycling process, producing a shift of sodium channels into the inactivated state from which recovery is delayed. Thus, ASDs effectively extend the 'refractory' period of the channel. As a result, these drugs produce a characteristic use- and frequency-dependent reduction in channel conductance, resulting in a limitation of repetitive neuronal firing, with little effect on the generation of single action potentials or on low frequency (<1 Hz) firing (Macdonald and Kelly, 1995). This is exemplified in experimental studies in which sustained repetitive action potential firing can be used as a bioassay for sodium channel blocking activity (Macdonald and McLean, 1986).

An extreme example of slow binding to the inactivated state is presented by lacosamide. Phenytoin and carbamazepine inhibit repetitive firing of cultured neurons in vitro within 100 milliseconds, whereas lacosamide, which also inhibits repetitive action potential firing, does so on a time scale of 1 second or more (Errington et al., 2008). This divergence was initially thought to be due to a preferential effect of lacosamide on slow inactivation of the sodium channel (Rogawski et al., 2015), an action that is also proposed for S-licarbazepine (Hebeisen et al., 2015). However, a more recent analysis suggests that the effects of lacosamide in this regard actually reflect very slow binding to the fast inactivated state of the channel (Jo and Bean, 2017). Since seizure discharges occur on the timescale of seconds, it is possible that the slow action of lacosamide might confer an even greater selectivity for seizure-related action potential firing than non-seizure-related firing, such that efficacy or tolerability might be improved. However, there is scant evidence that lacosamide has improved clinical effectiveness (Baulac et al., 2017).

In addition to effects on transient sodium currents, some ASDs can also block the persistent sodium current, which arises as a result of rare, late openings of $Na_V1.6$ channels in particular (Chatelier et al., 2010). Although the persistent current comprises only a small percentage of total sodium conductance in any single round of depolarisation, prolonged late openings can contribute significantly to a persistent depolarisation that is reminiscent of the paroxysmal depolarising shift which characterises epileptiform activity (Walker and Surges, 2016). There is evidence that phenytoin blocks the persistent sodium current and to a potentially greater degree than the transient current that underlies normal action potential generation (Segal and Douglas, 1997). Likewise, cenobamate, which, at the time of writing, has become the latest ASD to be approved by the FDA for use in focal-onset seizures, inhibits the persistent sodium current more potently than the transient sodium current (Nakamura et al., 2019), although it appears to have additional effects on $GABA_A$ receptors at marginally higher concentrations (discussed below). Other sodium channel blocking ASDs, including carbamazepine and topiramate, may also block the persistent sodium current, with a potency that can approximate or even exceed their effect on the transient sodium current (Sun et al., 2007). As such, inhibition of the persistent sodium current could contribute to the ability of these various agents to suppress sustained depolarisations while sparing single action potentials and low frequency firing.

2.2 Blockade of voltage-gated calcium channels

Voltage-gated calcium channels are involved in neuronal burst firing and are responsible for the control of neurotransmitter release at presynaptic nerve terminals. Like sodium channels, voltage-gated calcium channels comprise a single α1-subunit protein, typically 170-240 kDa, which again comprises four homologous domains each with six transmembrane segments (Catterall, 2000). Molecular studies have identified ten different α1-subunits ($Ca_V1.1$-1.4, $Ca_V2.1$-2.3, $Ca_V3.1$-3.3), at least seven of which are known to be expressed in mammalian brain (Trimmer and Rhodes, 2004). In addition, there are a number of accessory proteins, including β- and $\alpha 2\delta$-subunits, that modulate the function and cell-surface expression of the α1-subunit but which are not essential for basic channel functionality (Dolphin, 2012).

There are four main types of voltage-gated calcium channel in mammalian brain, commonly grouped into two classes on the basis of their biophysical properties and patterns of cellular expression (Catterall, 2000). L-type, P/Q-type and N-type belong to the class of high-voltage-activated calcium channels that respond to strong depolarisations and are involved in the processing of synaptic inputs at the somatodendritic level (L-type) and in presynaptic neurotransmitter release (P/Q- and N-type). The L-type channel comprises α1-subunits from the Ca_V1 family, while P/Q-type and N-type channels are formed from $Ca_V2.1$ and $Ca_V2.2$ α1-subunits, respectively (Trimmer and Rhodes, 2004). In contrast, the low-voltage-activated T-type calcium channel (comprising α1-subunits from the Ca_V3 family) opens in response to modest depolarisations at or below resting membrane potential, rapidly inactivates, and gives rise to transient (hence T-type) currents that participate in intrinsic oscillatory activity (Suzuki and Rogawski, 1989). The T-type channel is highly expressed on the soma and dendrites of thalamic relay and reticular neurons where it has been shown to underpin the rhythmic 3 Hz spike-wave discharges that are characteristic of absence seizures (McCormick and Contreras, 2001).

Voltage-gated calcium channels represent an important target for several ASDs. The efficacy of ethosuximide in absence epilepsy is believed to be mediated predominantly by blockade of T-type calcium channels in thalamocortical neurons, with preferential affinity for channels in the inactivated state (Coulter et al., 1989; Gomora et al., 2001), but there is also evidence that this drug can block the persistent sodium current and/or calcium-dependent potassium currents (Leresche et al., 1998). Zonisamide is also believed to block T-type calcium channels as one of several proposed mechanisms of action (Suzuki et al., 1992) and there is anecdotal evidence that valproate, another effective antiabsence agent, can also block this channel type (Broicher et al., 2007).

Gabapentin and pregabalin also interact with voltage-activated calcium channels but the role of calcium channels in the antiseizure mechanism of these drugs is uncertain. Although gabapentin was originally designed as a GABAmimetic that could freely cross the blood-brain barrier, it is now accepted that it and the related gabapentinoid pregabalin are devoid of GABAergic activity and instead bind with high affinity to $\alpha2\delta$-1 subunits of the voltage-gated calcium channel (Thorpe and Offord, 2010). This binding interaction is believed to account for the therapeutic activities of the drugs. The binding site on $\alpha2\delta$-1 for gabapentinoids has been modelled based on a recent cryo-electron microscopy structure (Kotev et al., 2018). It is presumed that binding of the gabapentinoids causes a conformational change in $\alpha2\delta$-1 that alters its association with other proteins. It has long been assumed that the primary role of $\alpha2\delta$-1 is as a partner of calcium channel $\alpha1$-subunits, and there is extensive evidence that $\alpha2\delta$-1 promotes insertion and retention of $\alpha1$-subunits in the plasma membrane (Hendrich et al., 2008; Dolphin, 2013). However, the binding interaction between $\alpha2\delta$-1 and $\alpha1$ is weak, and calcium currents in brain neurons are unaffected by knockout of $\alpha2\delta$-1. Moreover, it has not been possible to reliably show a robust effect of gabapentin and pregabalin on calcium channel currents, raising the question of the role of calcium channels in the mechanism of action of these drugs. Although inhibition of presynaptic calcium channels with a consequent reduction in release of excitatory neurotransmitter is an appealing mechanism to explain the antiseizure activity of gabapentinoids, the experimental evidence is not supportive. Nevertheless, there are studies that demonstrate an inhibition of excitatory synaptic potentials at brain synapses, but the mechanism is obscure (Cunningham et al., 2004; Dooley et al., 2007). Recent studies indicate that $\alpha2\delta$-1 associates with other proteins, including NMDA receptors (Chen et al., 2018b). While inhibition of NMDA receptors could contribute to the antiseizure activity of gabapentinoids, this is unlikely to be the sole activity of the drugs as their profile in animal seizure models and clinical activity does not correspond with that of NMDA receptor antagonists. Interactions of $\alpha2\delta$-1 with other as yet unidentified targets could conceivably play a role.

Other ASDs have less selective but perhaps more conventional inhibitory effects on specific types of high-voltage-activated calcium channel. Lamotrigine blocks N- and P/Q-type calcium channels on presynaptic nerve terminals (Wang et al., 1996), an effect which likely explains early evidence that the drug is able to reduce synaptic release of glutamate, and levetiracetam appears to exert a partial blockade of N-type calcium currents (Lukyanetz et al., 2002), suggesting an effect on an as yet unidentified sub-class of this channel type. Likewise, phenobarbital and topiramate can block L- and N-type calcium currents (ffrench-Mullen et al., 1993; Zhang et al., 2000), although their effects on calcium channels at therapeutic concentrations are modest compared to effects on other likely antiseizure mechanisms (Löscher and Rogawski, 2012), and other ASDs, including oxcarbazepine and felbamate, also have actions, albeit less well characterised, on high-voltage activated calcium channels (Stefani et al., 1995; Stefani et al., 1996).

2.3. Potentiation of voltage-gated potassium channels

Voltage-gated potassium channels are critical determinants of neuronal excitability, responsible for repolarising the cell membrane in the aftermath of action potential firing and regulating the balance between input and output in individual neurons. As a group, they are highly heterogeneous. More than 40 voltage-gated potassium channel α-subunits are recognised, most of which are structurally similar to a single domain of the α-subunit of voltage-gated sodium and calcium channels (Gutman et al., 2005). They are classified into 12 subfamilies (K_V1 to K_V12), with individual channels comprising four α-subunits from the same subfamily arranged around a central potassium ion pore, typically in a 'two plus two' configuration (Kuang et al., 2015). Two major functional classes of voltage-gated potassium channel are extensively described in the literature; A-type (mostly K_V4) channels that rapidly activate and inactivate, and delayed rectifier channels that open (after a short delay) in response to depolarisation and which do not fully inactivate (Christie, 1995). This latter class comprises K_V1 to K_V3 channels that are expressed on dendrites, axons and nerve terminals and which repolarise the neuronal cell membrane after action potential firing. This class also includes K_V7 channels that are expressed in the soma and axon initial segment and are responsible for the M-current, which determines the threshold and rate of neuronal firing and modulates the somatic response to dendritic inputs (Robbins, 2001). Mutations in the *KCNQ* genes, which encode K_V7 channels, are associated with a spectrum of seizure disorders ranging from benign familial neonatal convulsions to severe epileptic encephalopathies (Maljevic and Lerche, 2014).

Retigabine (known as ezogabine in the USA) is an ASD that exerts its effects by activation of the K_V7 class of voltage-gated potassium channels, is specific for channels containing $K_V7.2$ to $K_V7.5$ subunits, and has particular affinity for channel assemblies containing dimers of $K_V7.2/K_V7.3$ and $K_V7.3/K_V7.5$ subunits (Tatulian et al., 2001). These channels underlie the M-current in seizure-prone regions of the brain, such as cerebral cortex and hippocampus. Retigabine enhances the M-current, increasing the rate at which it is activated by depolarisation and decreasing the rate at which it is subsequently deactivated (Gunthorpe et al., 2012). It also enhances the M-current at resting membrane potential, hyperpolarising the cell membrane and reducing overall excitability of neurons. This effect of retigabine is mediated by binding of the drug within the pore of the channel. A single amino acid (Trp236) located in the activation gate of the K_V7 α-subunit protein is essential and all four subunits in the channel assembly must contain a tryptophan residue at position 236 for retigabine sensitivity (Schenzer et al., 2005). Retigabine was originally licensed in the USA and Europe in 2011 for the treatment of focal seizures in adults (Porter et al., 2012). Its use was later restricted due to the emergence of idiosyncratic adverse effects and although subsequently withdrawn by the manufacturer, there remains interest in the use of retigabine as a precision therapy in severe epileptic encephalopathies due to mutations in the *KCNQ* genes (Ihara et al., 2016).

3. Potentiation of inhibitory neurotransmission

GABA is the predominant inhibitory neurotransmitter in the mammalian central nervous system and is released at up to 40% of all synapses in the brain. GABA is synthesised from glutamate by the action of the enzyme glutamic acid decarboxylase (GAD) and, following release from nerve terminals, acts on both $GABA_A$ and $GABA_B$ receptors, with a net inhibitory effect.

The $GABA_A$ receptor is a ligand-gated ion channel and a member of the classical “Cys-loop” receptor family that comprise five independent protein subunits arranged around a central ion pore that is, in this case, permeable to chloride and bicarbonate ions (Olsen and Sieghart, 2009). Nineteen $GABA_A$ receptor subunits have been identified to date, sixteen in brain (α1-6, β1-3, γ1-3, δ, ε, θ, π) and three additional subunits in retina (ρ1-3), which come together as heteromeric pentamers to form functional channels (Sieghart, 1995). Heterogeneity in subunit composition suggests that countless thousands of $GABA_A$ receptors might potentially exist but, in reality, only a handful of channels appear to be expressed in mammalian brain, the most common configuration containing two α1-subunits, two β2-subunits, and one γ2-subunit (Baumann et al., 2002). $GABA_A$ receptors mediating transient, rapidly desensitising currents at the synapse (phasic inhibition) typically comprise two α-, two β-, and one γ2-subunit, whereas those at extrasynaptic sites and mediating long-lasting, slowly desensitising currents (tonic inhibition) preferentially contain α4- and α6-subunits and a δ-subunit in place of the γ2-subunit (Belelli et al., 2009). In contrast to the $GABA_A$ receptor, $GABA_B$ receptors are coupled, via a G-protein, to potassium channels that mediate slow hyperpolarisation of the postsynaptic membrane (Bowery, 1993). This receptor is also expressed on presynaptic nerve terminals where it acts as an autoreceptor, with activation limiting further GABA release.

GABA is removed from the synaptic cleft into nerve terminals and glial cells by a family of transporter proteins, encoded by members of the SLC6 gene family and denoted GAT-1, GAT-2, GAT-3, and BGT-1, that transport GABA down an electrochemical gradient driven by sodium and chloride ions (Borden, 1996). GAT-1 is the major GABA transporter expressed on both presynaptic nerve terminals and glial cells in cerebral cortex and hippocampus, with GAT-3 expression predominantly restricted to glia (Ribak et al., 1996; Lee et al., 2006). Following carrier-mediated re-uptake, GABA is either recycled into the readily releasable neurotransmitter pool or inactivated by conversion to succinic acid semialdehyde in a reaction catalysed by the mitochondrial enzyme GABA-transaminase.

3.1 Allosteric modulation of $GABA_A$ receptors

Binding of neurotransmitter GABA to $GABA_A$ receptors induces opening of the chloride ion channel that is intrinsic to the receptor. By contrast, ASDs that act on the $GABA_A$ receptor are largely positive allosteric modulators. They do not open the receptor in the absence of GABA, although barbiturates can do this at high concentrations (Rho et al., 1996), but rather increase the response to synaptically released GABA, thereby enhancing inhibitory neurotransmission (Czuczwar and Patsalos, 2001). While barbiturates (i.e., phenobarbital, primidone) and benzodiazepines (i.e., diazepam, lorazepam, clonazepam and clobazam) share this effect, they bind to distinct sites on the receptor complex, possess different subunit specificities, and differentially influence the opening of the chloride channel.

The five subunits of $GABA_A$ receptors are organised in a barrel-like fashion with subunits arranged like staves in a specific configuration, forming the central chloride ion pore. For example, the most abundant synaptic $GABA_A$ receptor isoform consisting of $(\alpha1)_2(\beta2)_2(\gamma2)_1$ has subunits arranged $^{-}\alpha1^{+}$-$^{-}\gamma2^{+}$-$^{-}\beta2^{+}$-$^{-}\alpha1^{+}$-$^{-}\beta2^{+}$ counter-clockwise when viewed from the extracellular space. Each subunit has two surfaces that contact neighbouring subunits; the interface surfaces are designated principal (+) and complementary (–). The last $\beta2$ subunit (+)-interface contacts the initial $\alpha1$ subunit (–)-interface to close the circle. Each $GABA_A$ receptor binds two molecules of GABA at sites that are situated at the two β^{+}-α^{-} subunit interfaces (Baumann et al., 2003). Benzodiazepine drugs also have a well-characterised binding site: one per receptor complex, at the α^{+}-$\gamma2^{-}$ subunit interface (Sigel and Buhr, 1997). Identification of the binding site for barbiturate drugs has been challenging, and to date all studies addressing this issue have investigated anaesthetic barbiturates (or analogs) and not phenobarbital, which is used in epilepsy therapy because it is less sedating at doses that confer antiseizure activity (Löscher and Rogawski, 2012). Recent studies indicate that barbiturates also bind at intramembrane subunit interfaces, which for these agents are γ^{+}-β^{-} and α^{+}-β^{-} (Chiara et al., 2013; Olsen, 2018) and at least one additional interface (Maldifassi et al., 2016). All $GABA_A$ receptors containing at least one α- and one β-subunit appear susceptible to allosteric activation by barbiturates, with only minor differences in relative sensitivity based on individual subunit composition (Hevers and Lüddens, 1998). Importantly, barbiturates act on δ-subunit containing extrasynaptic $GABA_A$ receptors that mediate tonic inhibition (Feng and Macdonald, 2010). Neurotransmitter GABA acts as a "partial agonist" on $\alpha\beta\delta$ $GABA_A$ receptors (low efficacy activation even at saturating concentrations) and GABA currents generated by these receptors are markedly enhanced by barbiturates. However, it remains to be proven that positive allosteric modulation of extrasynaptic $GABA_A$ receptors is a relevant antiseizure mechanism.

In contrast to barbiturates, benzodiazepines display a high degree of subunit selectivity, they do not activate $GABA_A$ receptors in the absence of GABA even at high concentrations, and they exclusively act on synaptic $GABA_A$ receptors. Benzodiazepine-sensitive $GABA_A$ receptors are typically comprised of two α-subunits (chosen from $\alpha1$, $\alpha2$, $\alpha3$ or $\alpha5$), two β-subunits (either $\beta2$ or $\beta3$), and a $\gamma2$ subunit, whereas the δ-subunit-containing $GABA_A$ receptors that mediate tonic inhibition at extrasynaptic sites, are insensitive to benzodiazepines, as are those containing $\alpha4$- and $\alpha6$-subunits (Farrant and Nusser, 2005; Sigel and Ernst, 2018). There are also functional distinctions between barbiturates and benzodiazepines, with the former increasing the duration of chloride channel opening in response to a given amount of GABA and the latter increasing the frequency of channel opening (Twyman et al., 1989).

Several other ASDs exert their effects, at least in part, by an allosteric action at the $GABA_A$ receptor. These include stiripentol, an orphan drug that is licensed for Dravet syndrome, which is able to positively modulate all $GABA_A$ receptor isoforms including those containing δ-subunits (Fisher, 2011), and which extends the duration of chloride channel opening in response to synaptically-released GABA in manner similar to that observed with barbiturates (Quilichini et al., 2006). Indeed, a recent study indicated that stiripentol binds with high affinity to the γ^+-β^- and α^+-β^- interfaces as do barbiturates (Jayakar et al., 2019). Felbamate and topiramate also promote GABA responses at the $GABA_A$ receptor (Rho et al., 1997; Simeone et al., 2006a; Simeone et al., 2006b; Simeone et al., 2011), as one of several mechanisms of action, but these effects do not appear to occur by binding at barbiturate interaction sites (Jayakar et al., 2019). Cenobamate, which contains the alkyl carbamate moiety as does felbamate and retigabine, has also been shown to be a weak positive allosteric modulator of $GABA_A$ receptors in hippocampal neurons, with effects on both phasic and tonic inhibitory currents and on recombinant synaptic and extrasynaptic $GABA_A$ receptor isoforms that do not appear to occur via an interaction with the benzodiazepine binding site (Sharma et al., 2019). Finally, levetiracetam also has effects at the $GABA_A$ receptor, indirectly influencing receptor function by blocking its negative allosteric modulation by β-carbolines and zinc (Rigo et al., 2002). The relevance of this action to the clinical activity of the drug is uncertain.

3.2 Modulation of GABA disposition

Vigabatrin and tiagabine are products of a rational drug discovery approach which was, in their cases, aimed at boosting inhibitory neurotransmission mediated by GABA (Löscher and Schmidt, 1994). Both drugs act by altering the disposition of GABA after it is released in the process of synaptic inhibition, albeit by different mechanisms.

Vigabatrin is an irreversible inhibitor of the mitochondrial enzyme GABA-transaminase, which is responsible for the catabolism of GABA (Jung et al., 1977). It causes a marked increase in whole brain GABA concentrations that outlast the presence of the drug and that are only restored to normal levels following the synthesis of new enzyme protein (Schechter et al., 1977). Interestingly, inhibition of GABA-transaminase appears to cause a paradoxical reduction in vesicular GABA content and a corresponding reduction in inhibitory postsynaptic potentials that are carried by phasic $GABA_A$ receptors (Overstreet and Westbrook, 2001). The anticonvulsant action of the drug is instead believed to be mediated by an increase in cytosolic GABA concentrations in presynaptic nerve terminals that leads to a reversal of the GABA transporter, spill-over of GABA into the extrasynaptic space, and activation of the tonic $GABA_A$ receptor current (Wu et al., 2003).

Unlike vigabatrin, tiagabine exerts its effects at synapses where it acts as a potent, selective and competitive inhibitor of GABA re-uptake (Krogsgaard-Larsen et al., 1987). The tiagabine molecule is based on nipecotic acid, a well-known experimental GABA transport inhibitor, coupled to a lipophilic side chain that facilitates blood-brain barrier penetration. It is selective for the GAT-1 GABA transporter, blocking both neuronal and glial GABA re-uptake, and its pharmacological effects mirror the regional distribution of this protein, with a more pronounced action in hippocampus and neocortex (Borden et al., 1994; Meldrum and Chapman, 1999). While vigabatrin results in a wholesale elevation in brain GABA concentration, tiagabine causes a transient prolongation in the presence of synaptically-released GABA within synapses. Functionally, this leads to an increase in inhibitory postsynaptic potentials mediated by phasic $GABA_A$ receptors but the potential for spill-over into extrasynaptic regions and activation of tonic $GABA_A$ receptor currents exists, particularly following sustained exposure to high concentrations of the drug (Schousboe et al., 2011).

Several other ASDs have also been reported to influence GABA disposition by either increasing synthesis or release of this neurotransmitter or by inhibiting its breakdown. This remains the single most convincing mechanism by which valproate exerts its anticonvulsant effects (Löscher, 2002). It has been reported to enhance the expression of glutamic acid decarboxylase, to promote the release of GABA from presynaptic terminals, and to prevent the catabolism of GABA by inhibition of GABA-transaminase (Löscher, 1999). There is also anecdotal evidence that valproate can act as a positive allosteric modulator at the $GABA_A$ receptor (Ticku and Davis, 1981). Other drugs with a proposed action on GABA disposition include topiramate and gabapentin, with much of the evidence in this regard derived from ^{1}H-magnetic resonance spectroscopy studies in human epilepsies (Kuzniecky et al., 2002). Efforts to replicate these findings experimentally have been largely unsuccessful however, questioning their validity and whether they represent true mechanisms of action of these drugs or simply epiphenomena of other CNS effects (Leach et al., 1997; Sills et al., 2000).

3.3 Inhibition of carbonic anhydrase

The acid-base balance and maintenance of local pH is critical to normal functioning of the nervous system. Various isoenzymes of carbonic anhydrase play an important role in this regard, eleven of which are expressed in brain (Ruusuvuori and Kaila, 2014). These enzymes are responsible for catalysing the bi-directional conversion of carbon dioxide and water to bicarbonate and hydrogen ions ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$). The forward reaction is rapid, whereas the rate of the reverse reaction is more modest. As a result, inhibition of carbonic anhydrase influences the latter more significantly, producing a localised acidosis and elevated bicarbonate ion concentrations (Heuser et al., 1975).

The bicarbonate gradient has an important influence on the function of $GABA_A$ receptors, which are permeable to both chloride and bicarbonate ions. The efflux of negatively charged bicarbonate ions via the $GABA_A$ receptor has a depolarising effect on the postsynaptic membrane which, under normal physiological conditions, is out-weighed by inward chloride ion currents that cause hyperpolarisation (Staley et al., 1995). However, during high-frequency activation of GABAergic synapses, the chloride gradient collapses, leading to depolarisations mediated by GABA that are dependent on bicarbonate ion flux and which have additionally been suggested to contribute to removal of the magnesium ion block of localised NMDA receptors (Staley et al., 1995). Inhibition of carbonic anhydrase diminishes the bicarbonate gradient and provides a degree of protection against this paradoxical $GABA_A$ receptor-mediated excitation and its downstream consequences (Hamidi and Avoli, 2015).

Acetazolamide is a classic carbonic anhydrase inhibitor that has been employed as an antiseizure agent with some success, particularly in paediatric epilepsies and in the treatment of catamenial epilepsy, but whose use is limited by the development of tolerance (Reiss and Oles, 1996). Topiramate and zonisamide share this mechanism of action but are significantly less potent than acetazolamide and have reportedly greater selectivity for individual isoenzymes; acetazolamide is a non-selective inhibitor whereas topiramate appears to inhibit CA-II and CA-IV alone (Dodgson et al., 2000). There is also evidence to suggest that lacosamide may inhibit carbonic anhydrase, but this finding requires further verification (Temperini et al., 2010). Inhibition of carbonic anhydrase can be considered as a supplementary rather than primary mechanism of action for these ASDs and the extent to which it contributes to the clinical activity of these compounds remains unclear.

4. Blockade of excitatory neurotransmission

Glutamate is the principal excitatory neurotransmitter in the mammalian brain. Following release from glutamatergic nerve terminals, it exerts its effects on three specific subtypes of ionotropic receptor in the postsynaptic membrane, designated according to their agonist specificities; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA) (Hollmann and Heinemann, 1994). Ionotropic glutamate receptors (iGluRs) are heteromeric tetramers constructed from four individual protein subunits in a dimer-of-dimers configuration (Tichelaar et al., 2004). In the mammalian brain there are four AMPA receptor subunits (GluA1-GluA4), five kainate receptor subunits (GluK1-GluK5) and seven NMDA receptor subunits (GluN1, GluN2A-GluN2D, GluN3A, GluN3B), although splice variants of several subunits add to the complexity (Traynelis et al., 2010). In addition to acting on iGluRs that mediate fast excitatory responses, synaptically-released glutamate also activates metabotropic glutamate receptors (mGluRs), which are G-protein-coupled receptors that control cellular excitability and other cellular processes via second messenger signalling on a longer time scale (Reiner and Levitz, 2018). Some mGluRs function similarly to $GABA_B$ receptors in that they act predominantly as autoreceptors on glutamatergic terminals, limiting glutamate release (Schoepp, 2001).

All iGluRs respond to glutamate binding by increasing cation conductance resulting in neuronal depolarisation. Most AMPA and kainate receptors are permeable only to sodium ions, although AMPA receptors that lack a GluA2 subunit also conduct calcium (Dingledine et al., 1999). In addition to serving as the main mediators of fast excitatory synaptic transmission in brain, AMPA receptors are also critical to seizure generation (Rogawski, 2013). In contrast, while activation of kainate receptors can induce seizures, these receptors do not appear to play a pivotal role as kainate receptor knockout does not impair seizure generation (Fritsch et al., 2014). NMDA receptors are freely permeable to both sodium and calcium ions and, owing to a voltage-dependent blockade by magnesium ions at resting membrane potential, are only activated during periods of prolonged depolarisation, as occurs during epileptiform discharges (Dingledine et al., 1999). Glutamate is removed from the synapse into nerve terminals and glial cells by a family of specific sodium-dependent transport proteins (EAAT1–EAAT5) and is inactivated by the enzymes glutamine synthetase (glial cells only) and glutamate dehydrogenase.

Despite many decades of intense effort across many CNS disease areas, there are only a handful of currently licensed drugs that possess a selective action at glutamate receptors. One of those is perampanel, an ASD that exerts its effects by non-competitive block of AMPA receptors (Rogawski and Hanada, 2013). It has no known effect on other receptor types, glutamate or otherwise. Perampanel binds to the AMPA receptor at a site on the extracellular domain of the channel protein, close to the interface with the phospholipid membrane, and distinct from the glutamate recognition site (Yelshanskaya et al., 2016). Binding of perampanel induces a conformational change in AMPA receptor subunits that limits their ability to translate agonist (i.e. glutamate) binding into channel opening (Yelshanskaya et al., 2016). The net result is to reduce fast excitatory neurotransmission and thereby limit seizure generation and the ability of seizure discharges to spread. Blocking the receptor that has primary responsibility for fast excitatory neurotransmission might be expected to have negative consequences in terms of tolerability. However, at therapeutic doses, perampanel is believed to block only a small proportion of the AMPA receptor current, sufficient to retard epileptiform discharges while sparing most normal synaptic transmission (Rogawski and Cavazos, 2020). Because of the critical role of AMPA receptors in brain function, perampanel has a low therapeutic window: increasing the dose even slightly can result in adverse neurological effects.

In addition to perampanel, several other ASDs exert their effects, in part, by an action on glutamatergic neurotransmission. Blockade of the NMDA subtype of glutamate receptor is believed to contribute to the pharmacological profile of felbamate (Rho et al., 1994) and topiramate has been shown to block the effects of kainate application in primary hippocampal neuron cultures, indicating inhibitory effects at either AMPA or kainate receptors (Gibbs et al., 2000). Levetiracetam inhibits AMPA-mediated currents in cortical neurons at therapeutic concentrations (Carunchio et al., 2007), and phenobarbital has also been reported to block AMPA receptors in a competitive manner, albeit at concentrations towards the upper end of its clinical range (Jin et al., 2010).

5. Modulation of neurotransmitter release

Several ASDs, most notably lamotrigine, have been reported to selectively reduce the release of glutamate from presynaptic nerve terminals (Leach et al., 1991). Although this phenomenon has been observed experimentally, it likely reflects an inhibitory action on presynaptic sodium and/or calcium channels rather than any specific effect on the synaptic vesicle release machinery in glutamatergic terminals. A more direct effect on neurotransmitter release may be produced by the ASD levetiracetam and its recently licensed analogue brivaracetam.

Levetiracetam was developed and licensed for the treatment of epilepsy with no clear indication of how it acts at the cellular level. A specific binding site for the drug in mammalian brain was later identified and determined to be synaptic vesicle protein 2A (SV2A) (Lynch et al., 2004). This protein is now considered to be the primary target of both levetiracetam and brivaracetam. Both drugs bind to SV2A, with brivaracetam being more potent and selective in this respect, and have little or no affinity for SV2B or SV2C, the other members of the SV2 protein family (Gillard et al., 2011). There is an striking correlation between SV2A binding affinity and the anticonvulsant efficacy of a series of levetiracetam analogues in audiogenic seizure sensitive mice, which strongly suggests that this is the site via which they exert their antiseizure effects (Kaminski et al., 2008). The anticonvulsant efficacy of levetiracetam is also diminished in heterozygous $SV2A^{+/-}$ mice (expression of SV2A protein reduced by 50%), which lends further support to the notion that SV2A is the primary target for seizure protection (Kaminski et al., 2009). However, despite intense investigation, the precise physiological role of SV2A is still unclear and it remains to be determined how drug binding influences SV2A.

SV2A belongs to the major facilitator superfamily of 12-transmembrane domain transporters, although no transport function has thus far been identified (Mendoza-Torreblanca et al., 2013). SV2A protein is highly expressed in presynaptic nerve terminals where it contributes to the complex protein interactions involved in synaptic vesicle release and recycling. It appears to interact with synaptotagmin, which acts as the calcium sensor in presynaptic terminals, and has been proposed to regulate the probability of vesicle fusion with the presynaptic membrane by altering sensitivity to calcium (Janz et al., 1999; Custer et al., 2006). Levetiracetam appears to enter nerve terminals via recycled synaptic vesicles, where it then binds to selected amino acids (Phe658, Gly659 and Val661) that lie within the 10th transmembrane domain of the SV2A molecule but it does not appear to cause a major conformational change in protein structure, suggesting a modest effect on protein function (Lynch et al., 2008). Exposure to levetiracetam limits release of both glutamate and GABA from rat brain slices in an activity-dependent manner, with greatest effect on rapidly-discharging neurons which would be consistent with selective suppression of epileptiform activity (Meehan et al., 2012). Homozygous SV2A knockout in mice leads to a lethal seizure phenotype, suggesting that the presence of the protein acts to retard seizure generation (Crowder et al., 1999). As such, it is assumed that levetiracetam and brivaracetam facilitate the action of SV2A but there is no data that unequivocally support this conclusion. Likewise, it remains unclear whether binding of the drugs to SV2A leads to altered packaging, trafficking, membrane fusion or recycling of vesicles within the nerve terminal.

6. Cannabinoids

Cannabidiol (CBD), a non-psychoactive plant-derived cannabinoid, was found empirically to be effective in the treatment of certain epileptic encephalopathies, including Dravet syndrome and Lennox-Gastaut syndrome as well tuberous sclerosis complex (TSC) (Hess et al., 2016; Chen et al., 2019). CBD exhibits broad-spectrum antiseizure activity in animal seizure models, although relatively high doses are required (Consroe et al., 1982; Jones et al., 2010; Klein et al., 2017). Unlike the structurally related cannabinoid Δ^9-tetrahydrocannabinol (THC), which acts as an agonist of CB1 (central nervous system) and CB2 (immune system) cannabinoid receptors, CBD is not a CB1 or CB2 receptor agonist. Moreover, whereas the CB1 receptor antagonist rimonabant blocks the antiseizure activity of THC, it does not block the antiseizure activity of CBD, confirming that the effect of CBD on seizures is not due to an action on brain CB1 receptors (Wallace et al., 2001). The basis of the antiseizure activity of CBD is unknown. Among the targets that have been proposed are G-protein coupled receptor GPR55, transient receptor potential cation channel TRPV1, voltage-gated sodium channels, and equilibrative nucleoside transporter ENT1. CBD is an antagonist of GPR55 (IC_{50}, 0.4 mM), which is an orphan G-protein coupled receptor activated by endocannabinoids and some plant-derived and synthetic cannabinoid ligands (Ryberg et al., 2007; Marichal-Cancino et al., 2017). Deletion of GPR55 in mice produces no conspicuous gross phenotypic, behavioural or pathological changes and there have been no mention of changes in seizure susceptibility, which would be expected if inhibition of GPR55 is an antiseizure mechanism (Wu et al., 2013; Bjursell et al., 2016). Nevertheless, GPR55 is expressed in brain regions relevant to epilepsy, including the dentate gyrus and other regions of the hippocampus where it is present in both interneurons and excitatory neurons (Balenga et al., 2011; Kaplan et al., 2017). CBD has demonstrated clinical efficacy in the treatment of seizures associated with Dravet syndrome, which is often caused by mutations in $Na_V1.1$ voltage-gated sodium channels that are predominantly expressed in inhibitory interneurons. Reduced sodium current in interneurons and impaired inhibitory function is believed to be the pathogenic mechanism in Dravet syndrome cases associated with haploinsufficiency of the *SCN1A* gene that encodes $Na_V1.1$ (Parihar and Ganesh, 2013). CBD (albeit at high doses) protects against thermally-induced seizures (modelling febrile seizures) in a $Scn1a^{+/-}$ mouse model of Dravet syndrome (Kaplan et al., 2017). Moreover, CBD was found to increase action potential generation in hippocampal GABAergic interneurons in $Scn1a^{+/-}$ mice, which in turn increased the frequency of inhibitory events in dentate granule cells. An antagonist of GPR55 occluded the action of CBD, raising the possibility that CBD may exert an antiseizure action in Dravet syndrome through effects on GPR55.

CBD has also been reported to be an agonist of TRPV1, a non-selective cation channel, which is predominantly expressed in nociceptive neurons of the peripheral nervous system but may also be expressed in brain regions relevant to epilepsy including the dentate gyrus of the hippocampus (Iannotti et al., 2014). CBD was found to activate and rapidly desensitize TRPV1 and to reduce epileptiform activity in hippocampal brain slices. A link between the agonist effect on TRPV1 and antiseizure activity was not established. Indeed, TRPV1 activation with capsaicin enhanced excitatory transmission in the dentate gyrus of mice with experimental temporal lobe epilepsy, suggesting that TRPV1 activation could be pro-epileptic (Bhaskaran and Smith, 2010). Moreover, knockout of TRPV1 did not markedly impact chemoconvulsant seizures in neonatal mice (Kong et al., 2014).

CBD has also been found in patch clamp recordings to be a nonselective inhibitor of recombinant voltage-gated sodium channels at concentrations that could be relevant therapeutically (Ghovanloo et al., 2018). Moreover, CBD appeared to stabilize the sodium channel inactivated state as is the case for conventional sodium channel blocking ASDs. Nonselective sodium channel blockers are well recognized to aggravate seizures in Dravet syndrome (Brunklaus et al., 2012) and are contraindicated in the condition (Wirrell et al., 2017). Therefore, it is noteworthy that in large-scale clinical trials conducted to support approval of CBD in the United States for the treatment of Lennox-Gastaut syndrome and Dravet syndrome there was a greater prevalence of seizure worsening when CBD was used in patients with Lennox-Gastaut syndrome who were not taking clobazam and in patients with Dravet syndrome who were not taking clobazam and stiripentol (Rogawski, 2019). The sodium channel blocking action of CBD could possibly account for the worsening, which seems to be masked by concomitant administration of a positive modulator of $GABA_A$ receptors. In clinical trials, CBD had reduced therapeutic efficacy when used in the absence of clobazam. While pharmacodynamic factors could contribute to the favourable interaction between CBD and clobazam, a pharmacokinetic drug-drug interaction almost certainly plays a role. CBD is an inhibitor of CYP2C19 and causes a marked (2.5 to 3-fold) increase in plasma concentrations of norclobazam, an active metabolite of clobazam (Geffrey et al., 2015; Rogawski, 2019).

An effect on adenosine dynamics is among the most plausible mechanisms proposed to explain the antiseizure activity of CBD. In studies of cannabinoid actions on immune function, it was found that CBD potently inhibits (IC_{50}, 0.12 mM) ENT1, one isoform of the most abundant family of mammalian plasma membrane transporters of nucleosides including adenosine (Carrier et al., 2006). ENT1, which acts as an equilibrative bidirectional transporter, is widely distributed throughout the body and is present in the brain. Block of ENT1 by CBD could theoretically enhance extracellular adenosine. Inasmuch as adenosine is well recognized to inhibit seizure mechanisms, this is a reasonable hypothesis to explain the antiseizure activity of CBD but no supporting evidence has as yet been presented.

7. Disease-specific mechanisms

7.1 mTORC1 signalling

In epilepsies caused by a specific genetically defined abnormality, a therapy that functionally reverses the molecular defect should prevent the occurrence of seizures and possibly also treat associated comorbidities. Everolimus, which is approved for the treatment of focal seizures associated with TSC, is such a disease-specific therapy.

Malformations of cortical development are a common cause of epileptic encephalopathies and pharmacoresistant seizures. Many of these epileptic encephalopathies are believed to be due to dysfunction in the mTOR (mechanistic target of rapamycin) signalling cascade (Jeong and Wong, 2018). mTOR is a protein kinase that is a central cell growth regulator (Kim and Guan, 2019). mTOR forms the catalytic subunit of mTORC1, which is a cytosolic protein complex that in addition to mTOR includes the core components Raptor (regulatory-associated protein of mTOR) and mLST8 (mammalian lethal with Sec13 protein 8) as well as certain inhibitory proteins. Drugs that inhibit mTORC1, such as rapamycin (sirolimus) and the rapalog everolimus, have various clinical roles including prevention of organ transplant rejection and slowing cancer growth and spread. Rapamycin and everolimus bind to the cyclophilin protein FKBP12, a peptidyl-prolyl isomerase (Houghton, 2010). The rapamycin-FKBP12 complex then allosterically inhibits mTORC1 by binding to mTOR (when it is associated with Raptor and MLST8).

Tuberous sclerosis is caused by loss-of-function mutations in the TSC1 gene encoding the protein hamartin or in the TSC2 gene encoding tuberin (Hasbani and Crino, 2018). The mutations lead to constitutive mTOR activation, resulting in abnormal cerebral cortical development with multiple focal structural malformations (Lasarge and Danzer, 2014). The substrate for the development of epilepsy is believed to be cortical tubers and peri-tuberal cortical tissue with dysmorphic neurons, giant cells, reactive astrocytes and disturbed cortical layering (Jeong and Wong, 2018). The precise basis for epileptogenesis in the presence of these diverse cellular abnormalities is not understood. However, the recognition that mTOR signalling pathway hyperactivity is the basis for the seizure disorder in TSC led to the investigation of mTOR inhibitors everolimus and sirolimus in clinical trials with favourable results (Curatolo et al., 2018). Apart from TSC, mTOR dysregulation has been implicated in a large spectrum of genetic and acquired epilepsies, particularly those associated with malformations of cortical development (Jeong and Wong, 2018). However, to date, there is no evidence that everolimus is effective in epilepsies other than those associated with TSC.

7.2 Lysosomal enzyme replacement

Neuronal ceroid lipofuscinoses (Batten disease) are a group of inherited disorders caused by deficiencies in lysosomal enzymes in which there is progressive intellectual and motor function deterioration with refractory seizures (Johnson et al., 2019). One of these conditions, neuronal ceroid lipofuscinosis type 2 (CLN2), is caused by lack of a functional tripeptidyl peptidase 1 (TPP-1) enzyme, which serves as a lysosomal exopeptidase that acts on a broad range of protein substrates. Individuals with CLN2 disease exhibit refractory myoclonic seizures, ataxia, developmental arrest and regression, central hypotonia with appendicular spasticity, and rapidly progressing motor decline. Symptomatic treatment is provided by cerliponase alfa, a recombinantly engineered human TPP-1 proenzyme delivered by intraventricular infusion that replaces the enzyme in the brain (Schulz et al., 2018). Cerliponase alfa is taken up by target cells in the brain and is translocated to the lysosomes through the cation independent mannose-6-phosphate receptor (M6P/IGF2 receptor). The proenzyme is activated in lysosomes and the activated proteolytic form cleaves tripeptides from the N-terminus of lysosomal proteins.

Cerliponase alfa treatment has been demonstrated to slow the progressive motor deterioration in CLN2 disease and improve survival (Schulz et al., 2018). There also appears to be improvement in seizures but one-half of children studied did exhibit seizures during treatment. In clinical trials, children remained on antiseizure medications and the long-term effect of the treatment on seizures is uncertain. EEG examinations showed new epileptiform activity suggesting continued disease progression.

8. Mechanisms in Nonepileptic Conditions

ASD are commonly used for the symptomatic treatment of diverse nonepileptic conditions, notably pain conditions, migraine, and many psychiatric disorders (Kaufman, 2011). In some cases, the mechanisms accounting for the antiseizure activity of these drugs are also relevant to their activity in nonepileptic conditions. For example, benzodiazepines are used in the treatment of anxiety and panic disorders, alcohol withdrawal, insomnia, and spasticity, and are also frequently used for sedation. All of these effects are due to the actions of benzodiazepines as positive allosteric modulators of synaptic $GABA_A$ receptors. Sodium channel blockade can explain the activity of carbamazepine and oxcarbazepine in trigeminal neuralgia (Di Stefano and Truini, 2017) and the antiarrhythmic activity (and cardiotoxicity) of phenytoin (Vaughan Williams, 1984).

The analgesic activity of gabapentinoids in the treatment of neuropathic pain likely results from an interaction with $\alpha 2\delta$-1 as has been proposed for their antiseizure effect (Chincholkar, 2018).

At the system level, the efficacy of these drugs in chronic pain is thought to relate to the depression of presynaptic excitatory input onto dorsal horn neurons through interactions with $\alpha 2\delta$-1, which is upregulated after injury (Rogawski and Löscher, 2004). In addition, gabapentinoids may influence descending facilitation and inhibition, may induce anti-inflammatory effects, and may influence cortical mechanisms mediating the affective components of pain. The interaction partners of $\alpha 2\delta$-1 that account for these diverse effects may be similar or different from those mediating the antiseizure actions.

In other instances, it is less clear that the antiseizure mechanism relates to the therapeutic actions in nonepileptic conditions. For example, there is no firm evidence that sodium channel blockade underlies the efficacy of sodium channel blocking ASDs, notably carbamazepine and lamotrigine, in the treatment of bipolar mania (Johannessen Landmark, 2008). Similarly, the cellular effects that account for the efficacy of valproate in bipolar disorder and in migraine (Rogawski and Löscher, 2004; Rosenberg, 2007) are as equally obscure, if not more so, as those that are responsible for its antiseizure activity. The mechanism of action of topiramate in migraine prophylaxis is also not understood.

9. Polytherapy and polypharmacology

An ever-improving understanding of the primary mechanisms by which ASDs exert their effects reignites interest in the concept of rational polytherapy in epilepsy. Although ~50% of people with epilepsy can expect to achieve good seizure control with ASD monotherapy, a small but significant proportion of individuals require treatment with two or more drugs (Kwan and Brodie, 2006). There has long been an interest in how to deploy ASDs in combination therapy so as to optimise efficacy and tolerability (Ferrendelli, 1995; Brodie and Sills, 2011). There is extensive evidence of synergism between drugs from studies in experimental animals (Czuczwar et al., 2009) but results in such studies have not translated into clinical practice. Combinations have therefore been selected based on clinical experience. Indeed, prior to the 1980s, the combination of phenytoin and phenobarbital was routinely used without much scientific justification. Today, the best accepted combination is that of valproate and lamotrigine, which appears to possess a mutually beneficial pharmacokinetic and pharmacodynamic interaction (Brodie and Yuen, 1997; Pisani et al., 1999). However, a fundamental understanding of the mechanistic basis of ASD synergy has been elusive (Jonker et al., 2007). There has been a longstanding belief that combining drugs with distinct mechanisms is preferable to combining drugs that act on the same target (Giussani and Beghi, 2013) but the evidence for this is mostly lacking (Deckers et al., 2000). There has, however, been some support from post-hoc subgroup analyses of clinical trial data in which subjects are categorized according to the mechanistic classification of their baseline ASDs. Analysis of the pivotal clinical trial data obtained in support of registration of the sodium channel blocking ASD lacosamide found that adjunctive use of lacosamide when one or more sodium channel blocking ASDs was a background medication resulted in less robust efficacy and greater adverse effects than when used in patients whose baseline regimen did not include a sodium channel blocker (Sake et al., 2010).

While much has been written about rational polypharmacy in epilepsy, it has also been recognized that a single drug molecule may exert more than one antiseizure action at therapeutic concentrations, thus exhibiting "polypharmacology" (Reddy and Zhang, 2013). The combined effects on persistent sodium currents and $GABA_A$ receptors that are observed with cenobamate (Nakamura et al., 2018; Sharma et al., 2019) may be an example of this phenomenon. There is some evidence that cenobamate offers a greater opportunity for seizure freedom in the treatment of focal-onset epilepsies than other ASDs (Krauss et al., 2020). Whether this will be confirmed with widespread use remains to be determined. If it is, the polypharmacology of cenobamate could be the key to its ability to overcome pharmacoresistance. A range of drugs including valproate, felbamate, topiramate, zonisamide, rufinamide, adrenocorticotrophin, and cannabidiol are listed in Table 1 as potentially having multiple mechanisms; in some cases, inclusion in the list is based on lack of understanding of the mechanism, whereas in others (e.g., felbamate, topiramate and zonisamide) there is credible evidence of polypharmacology.

10. Summary and conclusions

For much of the history of the drug treatment of epilepsy, only a limited group of agents (bromide, phenobarbital, phenytoin, primidone, ethosuximide, carbamazepine and valproate) were available to clinicians. A turning point occurred in 1989 with the licensing of vigabatrin in the United Kingdom and Ireland. The subsequent 30 years has seen an explosion in the number of small molecule ASDs approved by regulatory authorities throughout the world. Virtually all of these agents were identified by screening in animal models that are unbiased as to mechanism. While the new ASDs are chemically extremely diverse and while their mechanisms of action, to the extent known, are also relatively diverse, the overall outcome in terms of seizure freedom has not improved (Chen et al., 2018a). During this period, there have also been remarkable advances in our understanding of how ASDs affect excitability mechanisms at the cellular level. Unfortunately, this knowledge has not been successfully applied to the development of agents with better efficacy.

Even with the advances that have been made, our understanding of ASD mechanisms remains incomplete. Nowhere is this more evident than in the case of valproate, where more than 50 years after its first use in the treatment of epilepsy there is still debate as to which if any of the drug's diverse and often subtle cellular effects relate to clinical efficacy (Löscher, 2002). In this article, we have focused on the primary mechanism(s) of action of ASDs, where these are known. Many drugs used currently in the treatment of epilepsy have additional, less well-characterised pharmacological effects that manifest at therapeutic concentrations and that might contribute to the drug's overall clinical profile. It is also possible that these actions are pharmacologically demonstrable but not of clinical relevance. There is no sure fire way to determine whether a specific drug action is or is not contributory to clinical activity. Some such effects of uncertain relevance include enhancement of $GABA_A$-receptor conductance by carbamazepine and phenytoin (Granger et al., 1995), modulation of serotonergic (Dailey et al., 1997) and purinergic transmission (Marangos et al., 1987) by carbamazepine, and alterations in the brain concentrations and turnover of a range of amino acid neurotransmitters by valproate (Löscher, 1993).

While substantial attention has been directed to elucidating antiseizure mechanisms, the cellular actions that underlie the adverse effects of ASDs remain relatively unexplored. There is a tendency to assume that the mechanisms accounting for seizure protection are the same as those that are responsible for side effects. This may be true in some cases, i.e., dizziness, nystagmus and diplopia observed with sodium channel blocking ASDs are likely caused by inhibition of high-frequency action potential firing in vestibular and oculomotor circuits (Gittis et al., 2010). Likewise, the tendency of GABAergic ASDs to cause somnolence is likely due to the same actions that confer antiseizure effects: enhanced availability of GABA or positive allosteric modulation of $GABA_A$ receptors (Brohan and Goudra, 2017). However, there are many specific CNS-related adverse effects of individual ASDs, such as cognitive impairment caused by topiramate and aggressivity caused by levetiracetam and perampanel, that may or may not be attributable to the same mechanisms that are responsible for their antiseizure effects (Hansen et al., 2018). Moreover, it is noteworthy that systemic toxicities, including blood dyscrasias, hepatotoxicities, and hypersensitivity reactions occur with many ASDs as a result of drug actions unrelated to the therapeutic mechanisms of action (Leeder, 1998).

In recent decades, the science of epilepsy has seen dramatic progress as advances in genetics have led to an explosion in the understanding of the pathophysiological bases of certain rare epilepsy syndromes and epileptic encephalopathies. We are just now beginning to see the emergence of therapies that target the underlying disease mechanisms in these syndromes, exemplified by everolimus in the treatment of tuberous sclerosis-associated focal seizures. There is now cause for optimism that we are entering a new paradigm where it will be possible to engineer specific treatments for some genetically-defined epilepsies using disease-mechanism targeted small molecules, antisense, gene therapy with viral vectors, and other biological approaches. In fact, there is good reason to believe that in certain genetic syndromes, therapies personalized to an individual patient's specific mutation(s) will be possible. These therapies, or derivatives thereof, may ultimately prove to have utility in more common polygenic epilepsies, where the underlying pathophysiology is a result of complex genetic variation at multiple loci, but where a specific genetic variant nonetheless plays a contributory role. However, until the causes of the common epilepsies are better understood, most patients suffering from epilepsy are unlikely to reap the benefits of this technological revolution.

References

Balenga NA, Henstridge CM, Kargl J, Waldhoer M. Pharmacology, signaling and physiological relevance of the G protein-coupled receptor 55. Adv Pharmacol. 2011;62:251-77.

Baulac M, Rosenow F, Toledo M, Terada K, Li T, De Backer M, Werhahn KJ, Brock M. Efficacy, safety, and tolerability of lacosamide monotherapy versus controlled-release carbamazepine in patients with newly diagnosed epilepsy: a phase 3, randomised, double-blind, non-inferiority trial. Lancet Neurol. 2017 Jan;16(1):43-54.

Baumann SW, Baur R, Sigel E. Forced subunit assembly in $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors. Insight into the absolute arrangement. J Biol Chem. 2002 Nov 29;277(48):46020-5.

Baumann SW, Baur R, Sigel E. Individual properties of the two functional agonist sites in $GABA_A$ receptors. J Neurosci. 2003 Dec 3;23(35):11158-66.

Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW. Extrasynaptic $GABA_A$ receptors: form, pharmacology, and function. J Neurosci. 2009 Oct 14;29(41):12757-63.

Bhaskaran MD, Smith BN. Effects of TRPV1 activation on synaptic excitation in the dentate gyrus of a mouse model of temporal lobe epilepsy. Exp Neurol. 2010 Jun;223(2):529-36.

Bjursell M, Ryberg E, Wu T, Greasley PJ, Bohlooly-Y M, Hjorth S. Deletion of Gpr55 results in subtle effects on energy metabolism, motor activity and thermal pain sensation. PLoS One. 2016 Dec 12;11(12):e0167965.

Borden LA. GABA transporter heterogeneity: pharmacology and cellular localization. Neurochem Int. 1996 Oct;29(4):335-56.

Borden LA, Murali Dhar TG, Smith KE, Weinshank RL, Branchek TA, Gluchowski C. Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. Eur J Pharmacol. 1994 Oct 14;269(2):219-24.

Bowery NG. $GABA_B$ receptor pharmacology. Annu Rev Pharmacol Toxicol. 1993;33:109-47.

Brodie MJ, Sills GJ. Combining antiepileptic drugs--rational polytherapy? Seizure. 2011 Jun;20(5):369-75.

Brodie MJ, Yuen AW. Lamotrigine substitution study: evidence for synergism with sodium valproate? 105 Study Group. Epilepsy Res. 1997 Mar;26(3):423-32.

Brohan J, Goudra BG. The role of GABA receptor agonists in anesthesia and sedation. CNS Drugs. 2017 Oct;31(10):845-856.

Broicher T, Seidenbecher T, Meuth P, Munsch T, Meuth SG, Kanyshkova T, Pape HC, Budde T. T-current related effects of antiepileptic drugs and a Ca^{2+} channel antagonist on thalamic relay and local circuit interneurons in a rat model of absence epilepsy. Neuropharmacology. 2007 Sep;53(3):431-46.

Brunklaus A, Ellis R, Reavey E, Forbes GH, Zuberi SM. Prognostic, clinical and demographic features in *SCN1A* mutation-positive Dravet syndrome. Brain. 2012 Aug;135(Pt 8):2329-36.

Carrier EJ, Auchampach JA, Hillard CJ. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. Proc Natl Acad Sci U S A. 2006 May 16;103(20):7895-900.

Carunchio I, Pieri M, Ciotti MT, Albo F, Zona C. Modulation of AMPA receptors in cultured cortical neurons induced by the antiepileptic drug levetiracetam. Epilepsia. 2007 Apr;48(4):654-62.

Catterall WA. Cellular and molecular biology of voltage-gated sodium channels. Physiol Rev. 1992 Oct;72(4 Suppl):S15-48.

Catterall WA. Structure and regulation of voltage-gated Ca^{2+} channels. Annu Rev Cell Dev Biol. 2000;16:521-55.

Catterall WA. Forty years of sodium channels: structure, function, pharmacology, and epilepsy. Neurochem Res. 2017 Sep;42(9):2495-2504.

Catterall WA, Swanson TM. Structural basis for pharmacology of voltage-gated sodium and calcium channels. Mol Pharmacol. 2015 Jul;88(1):141-50.

Chatelier A, Zhao J, Bois P, Chahine M. Biophysical characterisation of the persistent sodium current of the Nav1.6 neuronal sodium channel: a single-channel analysis. Pflugers Arch. 2010 Jun;460(1):77-86.

Chen JW, Borgelt LM, Blackmer AB. Cannabidiol: A new hope for patients with Dravet or Lennox-Gastaut syndromes. Ann Pharmacother. 2019 Jun;53(6):603-611.

Chen Z, Brodie MJ, Liew D, Kwan P. Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs: A 30-year longitudinal cohort study. JAMA Neurol. 2018a Mar 1;75(3):279-286.

Chen J, Li L, Chen SR, Chen H, Xie JD, Sirrieh RE, MacLean DM, Zhang Y, Zhou MH, Jayaraman V, Pan HL. The α2δ-1-NMDA receptor complex Is critically involved in neuropathic pain development and gabapentin therapeutic actions. Cell Rep. 2018b Feb 27;22(9):2307-2321.

Chiara DC, Jayakar SS, Zhou X, Zhang X, Savechenkov PY, Bruzik KS, Miller KW, Cohen JB. Specificity of intersubunit general anesthetic-binding sites in the transmembrane domain of the human α1β3γ2 γ-aminobutyric acid type A ($GABA_A$) receptor. J Biol Chem. 2013 Jul 5;288(27):19343-57.

Chincholkar M. Analgesic mechanisms of gabapentinoids and effects in experimental pain models: a narrative review. Br J Anaesth. 2018 Jun;120(6):1315-1334.

Christie MJ. Molecular and functional diversity of K^+ channels. Clin Exp Pharmacol Physiol. 1995 Dec;22(12):944-51.

Consroe P, Benedito MA, Leite JR, Carlini EA, Mechoulam R. Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice. Eur J Pharmacol. 1982 Sep 24;83(3-4):293-8.

Coulter DA, Huguenard JR, Prince DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. Ann Neurol. 1989 Jun;25(6):582-93.

Crill WE. Persistent sodium current in mammalian central neurons. Annu Rev Physiol. 1996;58:349-62.

Crowder KM, Gunther JM, Jones TA, Hale BD, Zhang HZ, Peterson MR, Scheller RH, Chavkin C, Bajjalieh SM. Abnormal neurotransmission in mice lacking synaptic vesicle protein 2A (SV2A). Proc Natl Acad Sci U S A. 1999 Dec 21;96(26):15268-73.

Cunningham MO, Woodhall GL, Thompson SE, Dooley DJ, Jones RS. Dual effects of gabapentin and pregabalin on glutamate release at rat entorhinal synapses in vitro. Eur J Neurosci. 2004 Sep;20(6):1566-76.

Curatolo P, Moavero R, van Scheppingen J, Aronica E. mTOR dysregulation and tuberous sclerosis-related epilepsy. Expert Rev Neurother. 2018 Mar;18(3):185-201.

Custer KL, Austin NS, Sullivan JM, Bajjalieh SM. Synaptic vesicle protein 2 enhances release probability at quiescent synapses. J Neurosci. 2006 Jan 25;26(4):1303-13.

Czuczwar SJ, Patsalos PN. The new generation of GABA enhancers. Potential in the treatment of epilepsy. CNS Drugs. 2001;15(5):339-50.

Czuczwar SJ, Kaplanski J, Swiderska-Dziewit G, Gergont A, Kroczka S, Kacinski M. Pharmacodynamic interactions between antiepileptic drugs: preclinical data based on isobolography. Expert Opin Drug Metab Toxicol. 2009 Feb;5(2):131-6.

Dailey JW, Reith ME, Yan QS, Li MY, Jobe PC. Carbamazepine increases extracellular serotonin concentration: lack of antagonism by tetrodotoxin or zero Ca2+. Eur J Pharmacol. 1997 Jun 11;328(2-3):153-62.

Deckers CL, Czuczwar SJ, Hekster YA, Keyser A, Kubova H, Meinardi H, Patsalos PN, Renier WO, Van Rijn CM. Selection of antiepileptic drug polytherapy based on mechanisms of action: the evidence reviewed. Epilepsia. 2000 Nov;41(11):1364-74.

Di Stefano G, Truini A. Pharmacological treatment of trigeminal neuralgia. Expert Rev Neurother. 2017 Oct;17(10):1003-1011.

Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. Pharmacol Rev. 1999 Mar;51(1):7-61.

Dodgson SJ, Shank RP, Maryanoff BE. Topiramate as an inhibitor of carbonic anhydrase isoenzymes. Epilepsia. 2000;41 Suppl 1:S35-9.

Dolphin AC. Calcium channel auxiliary α2δ and β subunits: trafficking and one step beyond. Nat Rev Neurosci. 2012 Jul 18;13(8):542-55.

Dolphin AC. The α2δ subunits of voltage-gated calcium channels. Biochim Biophys Acta. 2013 Jul;1828(7):1541-9.

Dooley DJ, Taylor CP, Donevan S, Feltner D. Ca^{2+} channel $\alpha 2\delta$ ligands: novel modulators of neurotransmission. Trends Pharmacol Sci. 2007 Feb;28(2):75-82.

Errington AC, Stöhr T, Heers C, Lees G. The investigational anticonvulsant lacosamide selectively enhances slow inactivation of voltage-gated sodium channels. Mol Pharmacol. 2008 Jan;73(1):157-69.

Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. Nat Rev Neurosci. 2005 Mar;6(3):215-29.

Feng HJ, Macdonald RL. Barbiturates require the N terminus and first transmembrane domain of the δ subunit for enhancement of $\alpha 1\beta 3\delta$ $GABA_A$ receptor currents. J Biol Chem. 2010 Jul 30;285(31):23614-21.

Ferrendelli JA. Rational polypharmacy. Epilepsia. 1995;36 Suppl 2:S115-8.

Ffrench-Mullen JM, Barker JL, Rogawski MA. Calcium current block by (–)-pentobarbital, phenobarbital, and CHEB but not (+)-pentobarbital in acutely isolated hippocampal CA1 neurons: comparison with effects on GABA-activated Cl^- current. J Neurosci. 1993 Aug;13(8):3211-21.

Fisher JL. The effects of stiripentol on $GABA_A$ receptors. Epilepsia. 2011 Apr;52 Suppl 2:76-8.

Fritsch B, Reis J, Gasior M, Kaminski RM, Rogawski MA. Role of GluK1 kainate receptors in seizures, epileptic discharges, and epileptogenesis. J Neurosci. 2014 Apr 23;34(17):5765-75.

Geffrey AL, Pollack SF, Bruno PL, Thiele EA. Drug-drug interaction between clobazam and cannabidiol in children with refractory epilepsy. Epilepsia. 2015 Aug;56(8):1246-51.

Ghovanloo MR, Shuart NG, Mezeyova J, Dean RA, Ruben PC, Goodchild SJ. Inhibitory effects of cannabidiol on voltage-dependent sodium currents. J Biol Chem. 2018 Oct 26;293(43):16546-16558.

Gibbs JW 3rd, Sombati S, DeLorenzo RJ, Coulter DA. Cellular actions of topiramate: blockade of kainate-evoked inward currents in cultured hippocampal neurons. Epilepsia. 2000;41 Suppl 1:S10-6.

Gilchrist J, Dutton S, Diaz-Bustamante M, McPherson A, Olivares N, Kalia J, Escayg A, Bosmans F. Nav1.1 modulation by a novel triazole compound attenuates epileptic seizures in rodents. ACS Chem Biol. 2014 May 16;9(5):1204-12.

Gillard M, Fuks B, Leclercq K, Matagne A. Binding characteristics of brivaracetam, a selective, high affinity SV2A ligand in rat, mouse and human brain: relationship to anti-convulsant properties. Eur J Pharmacol. 2011 Aug 16;664(1-3):36-44.

Gittis AH, Moghadam SH, du Lac S. Mechanisms of sustained high firing rates in two classes of vestibular nucleus neurons: differential contributions of resurgent Na, Kv3, and BK currents. J Neurophysiol. 2010 Sep;104(3):1625-34.

Giussani G, Beghi E. Does mechanism of drug action matter to inform rational polytherapy in epilepsy? CNS Neurol Disord Drug Targets. 2013 May 1;12(3):426-35.

Gomora JC, Daud AN, Weiergräber M, Perez-Reyes E. Block of cloned human T-type calcium channels by succinimide antiepileptic drugs. Mol Pharmacol. 2001 Nov;60(5):1121-32.

Granger P, Biton B, Faure C, Vige X, Depoortere H, Graham D, Langer SZ, Scatton B, Avenet P. Modulation of the gamma-aminobutyric acid type A receptor by the antiepileptic drugs carbamazepine and phenytoin. Mol Pharmacol. 1995 Jun;47(6):1189-96.

Gunthorpe MJ, Large CH, Sankar R. The mechanism of action of retigabine (ezogabine), a first-in-class K^+ channel opener for the treatment of epilepsy. Epilepsia. 2012 Mar;53(3):412-24.

Gutman GA, Chandy KG, Grissmer S, Lazdunski M, McKinnon D, Pardo LA, Robertson GA, Rudy B, Sanguinetti MC, Stühmer W, Wang X. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. Pharmacol Rev. 2005 Dec;57(4):473-508.

Hamidi S, Avoli M. Carbonic anhydrase inhibition by acetazolamide reduces in vitro epileptiform synchronization. Neuropharmacology. 2015 Aug;95:377-87.

Hansen CC, Ljung H, Brodtkorb E, Reimers A. Mechanisms underlying aggressive behavior induced by antiepileptic drugs: Focus on topiramate, levetiracetam, and perampanel. Behav Neurol. 2018 Nov 15;2018:2064027.

Hasbani DM, Crino PB. Tuberous sclerosis complex. Handb Clin Neurol. 2018;148:813-822.

Hebeisen S, Pires N, Loureiro AI, Bonifácio MJ, Palma N, Whyment A, Spanswick D, Soares-da-Silva P. Eslicarbazepine and the enhancement of slow inactivation of voltage-gated sodium channels: a comparison with carbamazepine, oxcarbazepine and lacosamide. Neuropharmacology. 2015 Feb;89:122-35.

Hendrich J, Van Minh AT, Heblich F, Nieto-Rostro M, Watschinger K, Striessnig J, Wratten J, Davies A, Dolphin AC. Pharmacological disruption of calcium channel trafficking by the $\alpha_2\gamma$ ligand gabapentin. Proc Natl Acad Sci U S A. 2008 Mar 4;105(9):3628-33.

Hess EJ, Moody KA, Geffrey AL, Pollack SF, Skirvin LA, Bruno PL, Paolini JL, Thiele EA. Cannabidiol as a new treatment for drug-resistant epilepsy in tuberous sclerosis complex. Epilepsia. 2016 Oct;57(10):1617-1624.

Heuser D, Astrup J, Lassen NA, Betz BE. Brain carbonic acid acidosis after acetazolamide. Acta Physiol Scand. 1975 Mar;93(3):385-90.

Hevers W, Lüddens H. The diversity of $GABA_A$ receptors. Pharmacological and electrophysiological properties of $GABA_A$ channel subtypes. Mol Neurobiol. 1998 Aug;18(1):35-86.

Houghton PJ. Everolimus. Clin Cancer Res. 2010 Mar 1;16(5):1368-72.

Hollmann M, Heinemann S. Cloned glutamate receptors. Annu Rev Neurosci. 1994;17:31-108.

Hull JM, Isom LL. Voltage-gated sodium channel β subunits: The power outside the pore in brain development and disease. Neuropharmacology. 2018 Apr;132:43-57.

Iannotti FA, Hill CL, Leo A, Alhusaini A, Soubrane C, Mazzarella E, Russo E, Whalley BJ, Di Marzo V, Stephens GJ. Nonpsychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability. ACS Chem Neurosci. 2014 Nov 19;5(11):1131-41.

Ihara Y, Tomonoh Y, Deshimaru M, Zhang B, Uchida T, Ishii A, Hirose S. Retigabine, a Kv7.2/Kv7.3-channel opener, attenuates drug-induced seizures in knock-In mice harboring Kcnq2 mutations. PLoS One. 2016 Feb 24;11(2):e0150095.

Janz R, Goda Y, Geppert M, Missler M, Südhof TC. SV2A and SV2B function as redundant Ca^{2+} regulators in neurotransmitter release. Neuron. 1999 Dec;24(4):1003-16.

Jayakar SS, Zhou X, Chiara DC, Jarava-Barrera C, Savechenkov PY, Bruzik KS, Tortosa M, Miller KW, Cohen JB. Identifying drugs that bind selectively to intersubunit general anesthetic sites in the $\alpha1\beta3\gamma2$ $GABA_AR$ transmembrane Domain. Mol Pharmacol. 2019 Jun;95(6):615-628.

Jeong A, Wong M. Targeting the mammalian target of rapamycin for epileptic encephalopathies and malformations of cortical development. J Child Neurol. 2018 Jan;33(1):55-63.

Jin LJ, Schlesinger F, Song YP, Dengler R, Krampfl K. The interaction of the neuroprotective compounds riluzole and phenobarbital with AMPA-type glutamate receptors: a patch-clamp study. Pharmacology. 2010;85(1):54-62.

Jo S, Bean BP. Lacosamide inhibition of Nav1.7 voltage-gated sodium channels: slow binding to fast-inactivated states. Mol Pharmacol. 2017 Apr;91(4):277-286.

Johannessen Landmark C. Antiepileptic drugs in non-epilepsy disorders: relations between mechanisms of action and clinical efficacy. CNS Drugs. 2008;22(1):27-47.

Johnson TB, Cain JT, White KA, Ramirez-Montealegre D, Pearce DA, Weimer JM. Therapeutic landscape for Batten disease: current treatments and future prospects. Nat Rev Neurol. 2019 Mar;15(3):161-178.

Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ, Stephens GJ. Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. J Pharmacol Exp Ther. 2010 Feb;332(2):569-77.

Jonker DM, Voskuyl RA, Danhof M. Synergistic combinations of anticonvulsant agents: what is the evidence from animal experiments? Epilepsia. 2007 Mar;48(3):412-34.

Jung MJ, Lippert B, Metcalf BW, Böhlen P, Schechter PJ. γ-Vinyl GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor of GABA-T: effects on brain GABA metabolism in mice. J Neurochem. 1977 Nov;29(5):797-802.

Kaminski RM, Gillard M, Leclercq K, Hanon E, Lorent G, Dassesse D, Matagne A, Klitgaard H. Proepileptic phenotype of SV2A-deficient mice is associated with reduced anticonvulsant efficacy of levetiracetam. Epilepsia. 2009 Jul;50(7):1729-40.

Kaminski RM, Matagne A, Leclercq K, Gillard M, Michel P, Kenda B, Talaga P, Klitgaard H. SV2A protein is a broad-spectrum anticonvulsant target: functional correlation between protein binding and seizure protection in models of both partial and generalized epilepsy. Neuropharmacology. 2008 Mar;54(4):715-20.

Kaplan JS, Stella N, Catterall WA, Westenbroek RE. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. Proc Natl Acad Sci U S A. 2017 Oct 17;114(42):11229-11234.

Kaufman KR. Antiepileptic drugs in the treatment of psychiatric disorders. Epilepsy Behav. 2011 May;21(1):1-11.

Kim J, Guan KL. mTOR as a central hub of nutrient signalling and cell growth. Nat Cell Biol. 2019 Jan;21(1):63-71.

Klein BD, Jacobson CA, Metcalf CS, Smith MD, Wilcox KS, Hampson AJ, Kehne JH. Evaluation of cannabidiol in animal seizure models by the Epilepsy Therapy Screening Program (ETSP). Neurochem Res. 2017 Jul;42(7):1939-1948.

Kong WL, Min JW, Liu YL, Li JX, He XH, Peng BW. Role of TRPV1 in susceptibility to PTZ-induced seizure following repeated hyperthermia challenges in neonatal mice. Epilepsy Behav. 2014 Feb;31:276-80.

Kotev M, Pascual R, Almansa C, Guallar V, Soliva R. Pushing the limits of computational structure-based drug design with a cryo-EM structure: The Ca^{2+} channel α2δ-1 subunit as a test case. J Chem Inf Model. 2018 Aug 27;58(8):1707-1715.

Krauss GL, Klein P, Brandt C, Lee SK, Milanov I, Milovanovic M, Steinhoff BJ, Kamin M. Safety and efficacy of adjunctive cenobamate (YKP3089) in patients with uncontrolled focal seizures: a multicentre, double-blind, randomised,placebo-controlled, dose-response trial. Lancet Neurol. 2020 Jan;19(1):38-48.

Krogsgaard-Larsen P, Falch E, Larsson OM, Schousboe A. GABA uptake inhibitors: relevance to antiepileptic drug research. Epilepsy Res. 1987 Mar;1(2):77-93.

Kuang Q, Purhonen P, Hebert H. Structure of potassium channels. Cell Mol Life Sci. 2015 Oct;72(19):3677-93. doi: 10.1007/s00018-015-1948-5.

Kuo CC. A common anticonvulsant binding site for phenytoin, carbamazepine, and lamotrigine in neuronal Na^+ channels. Mol Pharmacol. 1998 Oct;54(4):712-21.

Kuo CC, Bean BP. Slow binding of phenytoin to inactivated sodium channels in rat hippocampal neurons. Mol Pharmacol. 1994 Oct;46(4):716-25.

Kuo CC, Chen RS, Lu L, Chen RC. Carbamazepine inhibition of neuronal Na^+ currents: quantitative distinction from phenytoin and possible therapeutic implications. Mol Pharmacol. 1997 Jun;51(6):1077-83.

Kuzniecky R, Ho S, Pan J, Martin R, Gilliam F, Faught E, Hetherington H. Modulation of cerebral GABA by topiramate, lamotrigine, and gabapentin in healthy adults. Neurology. 2002 Feb 12;58(3):368-72.

Kwan P, Brodie MJ. Combination therapy in epilepsy: when and what to use. Drugs. 2006;66(14):1817-29.

Lasarge CL, Danzer SC. Mechanisms regulating neuronal excitability and seizure development following mTOR pathway hyperactivation. Front Mol Neurosci. 2014 Mar 14;7:18.

Leach JP, Sills GJ, Butler E, Forrest G, Thompson GG, Brodie MJ. Neurochemical actions of gabapentin in mouse brain. Epilepsy Res. 1997 Jun;27(3):175-80.

Leach MJ, Baxter MG, Critchley MA. Neurochemical and behavioral aspects of lamotrigine. Epilepsia. 1991;32 Suppl 2:S4-8.

Lee TS, Bjørnsen LP, Paz C, Kim JH, Spencer SS, Spencer DD, Eid T, de Lanerolle NC. GAT1 and GAT3 expression are differently localized in the human epileptogenic hippocampus. Acta Neuropathol. 2006 Apr;111(4):351-63.

Leeder JS. Mechanisms of idiosyncratic hypersensitivity reactions to antiepileptic drugs. Epilepsia. 1998;39 Suppl 7:S8-16.

Leresche N, Parri HR, Erdemli G, Guyon A, Turner JP, Williams SR, Asprodini E, Crunelli V. On the action of the anti-absence drug ethosuximide in the rat and cat thalamus. J Neurosci. 1998 Jul 1;18(13):4842-53.

Löscher W. Effects of the antiepileptic drug valproate on metabolism and function of inhibitory and excitatory amino acids in the brain. Neurochem Res. 1993 Apr;18(4):485-502.

Löscher W. Valproate: a reappraisal of its pharmacodynamic properties and mechanisms of action. Prog Neurobiol. 1999 May;58(1):31-59.

Löscher W. Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. CNS Drugs. 2002;16(10):669-94.

Löscher W, Rogawski MA. How theories evolved concerning the mechanism of action of barbiturates. Epilepsia. 2012 Dec;53 Suppl 8:12-25.

Löscher W, Schmidt D. Strategies in antiepileptic drug development: is rational drug design superior to random screening and structural variation? Epilepsy Res. 1994 Feb;17(2):95-134.

Lukyanetz EA, Shkryl VM, Kostyuk PG. Selective blockade of N-type calcium channels by levetiracetam. Epilepsia. 2002 Jan;43(1):9-18.

Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B. The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proc Natl Acad Sci U S A. 2004 Jun 29;101(26):9861-6.

Lynch BA, Matagne A, Brännström A, von Euler A, Jansson M, Hauzenberger E, Söderhäll JA. Visualization of SV2A conformations in situ by the use of Protein Tomography. Biochem Biophys Res Commun. 2008 Oct 31;375(4):491-5.

Macdonald RL, Kelly KM. Antiepileptic drug mechanisms of action. Epilepsia. 1995;36 Suppl 2:S2-12.

Macdonald RL, McLean MJ. Anticonvulsant drugs: mechanisms of action. Adv Neurol. 1986;44:713-36.

Maldifassi MC, Baur R, Sigel E. Functional sites involved in modulation of the $GABA_A$ receptor channel by the intravenous anesthetics propofol, etomidate and pentobarbital. Neuropharmacology. 2016 Jun;105:207-214.

Maljevic S, Lerche H. Potassium channel genes and benign familial neonatal epilepsy. Prog Brain Res. 2014;213:17-53.

Mantegazza M, Curia G, Biagini G, Ragsdale DS, Avoli M. Voltage-gated sodium channels as therapeutic targets in epilepsy and other neurological disorders. Lancet Neurol. 2010 Apr;9(4):413-24.

Marangos PJ, Patel J, Smith KD, Post RM. Adenosine antagonist properties of carbamazepine. Epilepsia. 1987 Jul-Aug;28(4):387-94.

Marichal-Cancino BA, Fajardo-Valdez A, Ruiz-Contreras AE, Mendez-Díaz M, Prospero-García O. Advances in the physiology of GPR55 in the central nervous system. Curr Neuropharmacol. 2017;15(5):771-778.

McCormick DA, Contreras D. On the cellular and network bases of epileptic seizures. Annu Rev Physiol. 2001;63:815-46.

Meehan AL, Yang X, Yuan LL, Rothman SM. Levetiracetam has an activity-dependent effect on inhibitory transmission. Epilepsia. 2012 Mar;53(3):469-76.

Meldrum BS, Chapman AG. Basic mechanisms of gabitril (tiagabine) and future potential developments. Epilepsia. 1999;40 Suppl 9:S2-6.

Mendoza-Torreblanca JG, Vanoye-Carlo A, Phillips-Farfán BV, Carmona-Aparicio L, Gómez-Lira G. Synaptic vesicle protein 2A: basic facts and role in synaptic function. Eur J Neurosci. 2013 Dec;38(11):3529-39.

Nakamura M, Cho JH, Shin H, Jang IS. Effects of cenobamate (YKP3089), a newly developed anti-epileptic drug, on voltage-gated sodium channels in rat hippocampal CA3 neurons. Eur J Pharmacol. 2019 Jul 15;855:175-182.

Olsen RW. $GABA_A$ receptor: Positive and negative allosteric modulators. Neuropharmacology. 2018 Jul 1;136(Pt A):10-22.

Olsen RW, Sieghart W. $GABA_A$ receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology. 2009 Jan;56(1):141-8.

Overstreet LS, Westbrook GL. Paradoxical reduction of synaptic inhibition by vigabatrin. J Neurophysiol. 2001 Aug;86(2):596-603.

Parihar R, Ganesh S. The SCN1A gene variants and epileptic encephalopathies. J Hum Genet. 2013 Sep;58(9):573-80.

Pisani F, Oteri G, Russo MF, Di Perri R, Perucca E, Richens A. The efficacy of valproate-lamotrigine comedication in refractory complex partial seizures: evidence for a pharmacodynamic interaction. Epilepsia. 1999 Aug;40(8):1141-6.

Porter RJ, Burdette DE, Gil-Nagel A, Hall ST, White R, Shaikh S, DeRossett SE. Retigabine as adjunctive therapy in adults with partial-onset seizures: integrated analysis of three pivotal controlled trials. Epilepsy Res. 2012 Aug;101(1-2):103-12.

Prakriya M, Mennerick S. Selective depression of low-release probability excitatory synapses by sodium channel blockers. Neuron. 2000 Jun;26(3):671-82.

Quilichini PP, Chiron C, Ben-Ari Y, Gozlan H. Stiripentol, a putative antiepileptic drug, enhances the duration of opening of $GABA_A$ receptor channels. Epilepsia. 2006 Apr;47(4):704-16.

Ragsdale DS, Scheuer T, Catterall WA. Frequency and voltage-dependent inhibition of type IIA Na^+ channels, expressed in a mammalian cell line, by local anesthetic, antiarrhythmic, and anticonvulsant drugs. Mol Pharmacol. 1991 Nov;40(5):756-65.

Reddy AS, Zhang S. Polypharmacology: drug discovery for the future. Expert Rev Clin Pharmacol. 2013 Jan;6(1):41-7.

Reiner A, Levitz J. Glutamatergic signaling in the central nervous system: Ionotropic and metabotropic receptors in concert. Neuron. 2018 Jun 27;98(6):1080-1098.

Reiss WG, Oles KS. Acetazolamide in the treatment of seizures. Ann Pharmacother. 1996 May;30(5):514-9.

Rho JM, Donevan SD, Rogawski MA. Mechanism of action of the anticonvulsant felbamate: opposing effects on N-methyl-D-aspartate and γ-aminobutyric acid A receptors. Ann Neurol. 1994 Feb;35(2):229-34.

Rho JM, Donevan SD, Rogawski MA. Direct activation of $GABA_A$ receptors by barbiturates in cultured rat hippocampal neurons. J Physiol. 1996 Dec 1;497 (Pt 2):509-22.

Rho JM, Donevan SD, Rogawski MA. Barbiturate-like actions of the propanediol dicarbamates felbamate and meprobamate. J Pharmacol Exp Ther. 1997 Mar;280(3):1383-91.

Ribak CE, Tong WM, Brecha NC. GABA plasma membrane transporters, GAT-1 and GAT-3, display different distributions in the rat hippocampus. J Comp Neurol. 1996 Apr 15;367(4):595-606.

Rigo JM, Hans G, Nguyen L, Rocher V, Belachew S, Malgrange B, Leprince P, Moonen G, Selak I, Matagne A, Klitgaard H. The anti-epileptic drug levetiracetam reverses the inhibition by negative allosteric modulators of neuronal GABA- and glycine-gated currents. Br J Pharmacol. 2002 Jul;136(5):659-72.

Robbins J. KCNQ potassium channels: physiology, pathophysiology, and pharmacology. Pharmacol Ther. 2001 Apr;90(1):1-19.

Rogawski MA. AMPA receptors as a molecular target in epilepsy therapy. Acta Neurol Scand Suppl. 2013;(197):9-18.

Rogawski MA. Reduced efficacy and risk of seizure aggravation when cannabidiol is used without clobazam. Epilepsy Behav. 2019 Sep 13:106506.

Rogawski MA, Cavazos JE. Mechanisms of action of antiseizure medications. In: Wyllie's Treatment of Epilepsy: Principles and Practice, 7th ed. (Wyllie E, ed) Philadelphia, PA: Wolters Kluwer 2020, in press.

Rogawski MA, Hanada T. Preclinical pharmacology of perampanel, a selective non-competitive AMPA receptor antagonist. Acta Neurol Scand Suppl. 2013;(197):19-24.

Rogawski MA, Löscher W. The neurobiology of antiepileptic drugs for the treatment of nonepileptic conditions. Nat Med. 2004 Jul;10(7):685-92.

Rogawski MA, Tofighy A, White HS, Matagne A, Wolff C. Current understanding of the mechanism of action of the antiepileptic drug lacosamide. Epilepsy Res. 2015 Feb;110:189-205.

Rosenberg G. The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees? Cell Mol Life Sci. 2007 Aug;64(16):2090-103.

Ruusuvuori E, Kaila K. Carbonic anhydrases and brain pH in the control of neuronal excitability. Subcell Biochem. 2014;75:271-90.

Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol. 2007 Dec;152(7):1092-101.

Sake JK, Hebert D, Isojärvi J, Doty P, De Backer M, Davies K, Eggert-Formella A, Zackheim J. A pooled analysis of lacosamide clinical trial data grouped by mechanism of action of concomitant antiepileptic drugs. CNS Drugs. 2010 Dec;24(12):1055-68.

Schechter PJ, Tranier Y, Jung MJ, Böhlen P. Audiogenic seizure protection by elevated brain GABA concentration in mice: effects of γ-acetylenic GABA and γ-vinyl GABA, two irreversible GABA-T inhibitors. Eur J Pharmacol. 1977 Oct 15;45(4):319-28.

Schenzer A, Friedrich T, Pusch M, Saftig P, Jentsch TJ, Grötzinger J, Schwake M. Molecular determinants of KCNQ (Kv7) K^+ channel sensitivity to the anticonvulsant retigabine. J Neurosci. 2005 May 18;25(20):5051-60.

Schoepp DD. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J Pharmacol Exp Ther. 2001 Oct;299(1):12-20.

Schousboe A, Madsen KK, White HS. GABA transport inhibitors and seizure protection: the past and future. Future Med Chem. 2011 Feb;3(2):183-7.

Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, Dyke JP, Cahan H, Slasor P, Jacoby D, Kohlschütter A; CLN2 Study Group. Study of intraventricular cerliponase alfa for CLN2 disease. N Engl J Med. 2018 May 17;378(20):1898-1907.

Schwarz JR, Grigat G. Phenytoin and carbamazepine: potential- and frequency-dependent block of Na currents in mammalian myelinated nerve fibers. Epilepsia. 1989 May-Jun;30(3):286-94.

Segal MM, Douglas AF. Late sodium channel openings underlying epileptiform activity are preferentially diminished by the anticonvulsant phenytoin. J Neurophysiol. 1997 Jun;77(6):3021-34.

Sharma R, Song WS, Nakamura M, Neupane C, Shin H, Melnick SM, Glenn KJ, Jang I-S, Kim M-H, Park JB. Effects of cenobamate on GABA-A receptor modulation. Neurology. 2019 Apr; 92 (15 Supplement) P1.5-033.

Sieghart W. Structure and pharmacology of γ-aminobutyric acid A receptor subtypes. Pharmacol Rev. 1995 Jun;47(2):181-234.

Sigel E, Buhr A. The benzodiazepine binding site of $GABA_A$ receptors. Trends Pharmacol Sci. 1997 Nov;18(11):425-9.

Sigel E, Ernst M. The benzodiazepine binding sites of $GABA_A$ receptors. Trends Pharmacol Sci. 2018 Jul;39(7):659-671.

Sills GJ, Leach JP, Kilpatrick WS, Fraser CM, Thompson GG, Brodie MJ. Concentration-effect studies with topiramate on selected enzymes and intermediates of the GABA shunt. Epilepsia. 2000;41 Suppl 1:S30-4.

Silva J. Slow inactivation of Na^+ channels. Handb Exp Pharmacol. 2014;221:33-49.

Simeone TA, Otto JF, Wilcox KS, White HS. Felbamate is a subunit selective modulator of recombinant γ-aminobutyric acid type A receptors expressed in *Xenopus oocytes*. Eur J Pharmacol. 2006a Dec 15;552(1-3):31-5.

Simeone TA, Wilcox KS, White HS. Subunit selectivity of topiramate modulation of heteromeric $GABA_A$ receptors. Neuropharmacology. 2006b Jun;50(7):845-57.

Simeone TA, Wilcox KS, White HS. Topiramate modulation of β_1- and β_3-homomeric $GABA_A$ receptors. Pharmacol Res. 2011 Jul;64(1):44-52.

Stafstrom CE. Persistent sodium current and its role in epilepsy. Epilepsy Curr. 2007 Jan-Feb;7(1):15-22.

Staley KJ, Soldo BL, Proctor WR. Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. Science. 1995 Aug 18;269(5226):977-81.

Stefani A, Calabresi P, Pisani A, Mercuri NB, Siniscalchi A, Bernardi G. Felbamate inhibits dihydropyridine-sensitive calcium channels in central neurons. J Pharmacol Exp Ther. 1996 Apr;277(1):121-7.

Stefani A, Pisani A, De Murtas M, Mercuri NB, Marciani MG, Calabresi P. Action of GP 47779, the active metabolite of oxcarbazepine, on the corticostriatal system. II. Modulation of high-voltage-activated calcium currents. Epilepsia. 1995 Oct;36(10):997-1002.

Sun GC, Werkman TR, Battefeld A, Clare JJ, Wadman WJ. Carbamazepine and topiramate modulation of transient and persistent sodium currents studied in HEK293 cells expressing the $Na_v1.3$ α-subunit. Epilepsia. 2007 Apr;48(4):774-782.

Suzuki S, Kawakami K, Nishimura S, Watanabe Y, Yagi K, Seino M, Miyamoto K. Zonisamide blocks T-type calcium channel in cultured neurons of rat cerebral cortex. Epilepsy Res. 1992 Jun;12(1):21-7.

Suzuki S, Rogawski MA. T-type calcium channels mediate the transition between tonic and phasic firing in thalamic neurons. Proc Natl Acad Sci U S A. 1989 Sep;86(18):7228-32.

Tatulian L, Delmas P, Abogadie FC, Brown DA. Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. J Neurosci. 2001 Aug 1;21(15):5535-45.

Temperini C, Innocenti A, Scozzafava A, Parkkila S, Supuran CT. The coumarin-binding site in carbonic anhydrase accommodates structurally diverse inhibitors: the antiepileptic lacosamide as an example and lead molecule for novel classes of carbonic anhydrase inhibitors. J Med Chem. 2010 Jan 28;53(2):850-4.

Thorpe AJ, Offord J. The alpha2-delta protein: an auxiliary subunit of voltage-dependent calcium channels as a recognized drug target. Curr Opin Investig Drugs. 2010 Jul;11(7):761-70.

Tichelaar W, Safferling M, Keinänen K, Stark H, Madden DR. The Three-dimensional Structure of an Ionotropic Glutamate Receptor Reveals a Dimer-of-dimers Assembly. J Mol Biol. 2004 Nov 19;344(2):435-42.

Ticku MK, Davis WC. Effect of valproic acid on [^{3}H]diazepam and [^{3}H]dihydropicrotoxinin binding sites at the benzodiazepine-GABA receptor-ionophore complex. Brain Res. 1981 Oct 26;223(1):218-22.

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev. 2010 Sep;62(3):405-96.

Trimmer JS, Rhodes KJ. Localization of voltage-gated ion channels in mammalian brain. Annu Rev Physiol. 2004;66:477-519.

Twyman RE, Rogers CJ, Macdonald RL. Differential regulation of γ-aminobutyric acid receptor channels by diazepam and phenobarbital. Ann Neurol. 1989 Mar;25(3):213-20.

Vaughan Williams EM. A classification of antiarrhythmic actions reassessed after a decade of new drugs. J Clin Pharmacol. 1984 Apr;24(4):129-47.

Walker MC, Surges R. Mechanisms of antiepileptic drug action. In: The Treatment of Epilepsy, 4th edition; eds Shorvon S, Perucca P, Engel Jr J; Wiley Blackwell, Chichester, UK, 2016; pp 75-91.

Wallace MJ, Wiley JL, Martin BR, DeLorenzo RJ. Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects. Eur J Pharmacol. 2001 Sep 28;428(1):51-7.

Wang SJ, Huang CC, Hsu KS, Tsai JJ, Gean PW. Inhibition of N-type calcium currents by lamotrigine in rat amygdalar neurones. Neuroreport. 1996 Nov 25;7(18):3037-40.

Wang J, Ou SW, Wang YJ. Distribution and function of voltage-gated sodium channels in the nervous system. Channels (Austin). 2017 Nov 2;11(6):534-554.

Whitaker WR, Clare JJ, Powell AJ, Chen YH, Faull RL, Emson PC. Distribution of voltage-gated sodium channel alpha-subunit and beta-subunit mRNAs in human hippocampal formation, cortex, and cerebellum. J Comp Neurol. 2000 Jun 19;422(1):123-39.

Wirrell EC, Laux L, Donner E, Jette N, Knupp K, Meskis MA, Miller I, Sullivan J, Welborn M, Berg AT. Optimizing the Diagnosis and Management of Dravet Syndrome: Recommendations From a North American Consensus Panel. Pediatr Neurol. 2017 Mar;68:18-34.e3.

Wu CS, Chen H, Sun H, Zhu J, Jew CP, Wager-Miller J, Straiker A, Spencer C, Bradshaw H, Mackie K, Lu HC. GPR55, a G-protein coupled receptor for lysophosphatidylinositol, plays a role in motor coordination. PLoS One. 2013;8(4):e60314.

Wu Y, Wang W, Richerson GB. Vigabatrin induces tonic inhibition via GABA transporter reversal without increasing vesicular GABA release. J Neurophysiol. 2003 Apr;89(4):2021-34.

Yelshanskaya MV, Singh AK, Sampson JM, Narangoda C, Kurnikova M, Sobolevsky AI. Structural bases of noncompetitive inhibition of AMPA-subtype ionotropic glutamate receptors by antiepileptic Drugs. Neuron. 2016 Sep 21;91(6):1305-1315.

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

Table 1. Molecular targets of clinically used antiseizure drugs

Molecular Target	**Antiseizure Drugs That Act on Target**
Voltage-gated ion channels	
Voltage-gated sodium channels	phenytoin, fosphenytoin[1], carbamazepine, oxcarbazepine[2], eslicarbazepine acetate[3], lamotrigine, lacosamide, cenobamate; possibly, rufinamide, topiramate, zonisamide
Voltage-gated calcium channels	ethosuximide
Voltage-gated potassium channels	retigabine (ezogabine)
GABA inhibition	
$GABA_A$ receptors	phenobarbital, primidone, benzodiazepines including diazepam, lorazepam, clonazepam, midazolam, clobazam; stiripentol; possibly, topiramate, felbamate, cenobamate, retigabine (ezogabine)
GAT-1 GABA transporter	tiagabine
GABA transaminase	vigabatrin
Carbonic anhydrase inhibition	acetazolamide, topiramate, zonisamide; possibly lacosamide
Synaptic release machinery	
SV2A	levetiracetam, brivaracetam
$\alpha 2\delta$	gabapentin, gabapentin enacarbil[4], pregabalin
Ionotropic glutamate receptors	
AMPA receptor	perampanel
Disease specific	
mTORC1 signaling	everolimus
Lysosomal enzyme replacement	cerliponase alfa (recombinant tripeptidyl peptidase 1)
Mixed/unknown	valproate, felbamate, cenobamate, topiramate, zonisamide, rufinamide, adrenocorticotrophin, cannabidiol

[1]Fosphenytoin is a prodrug for phenytoin, [2]Oxcarbazepine serves largely as a prodrug for licarbazepine, mainly S-licarbazepine; [3]Eslicarbarbazepine acetate is a prodrug for S-licarbazepine; [4]Gabapentin enacarbil is a prodrug for gabapentin

Table adapted from Rogawski and Cavazos (2020).