Title

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Publication Date

2008-07-17

Peer reviewed

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[Published in slightly abridged form, without figure, in *Current Neurology and Neuroscience Reports* **8:**345–352, 2008]

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Abstract

Many currently prescribed antiepileptic drugs (AEDs) act via voltage-gated sodium channels, through effects on γ-aminobutyric acid–mediated inhibition, or via voltage-gated calcium channels. Some newer AEDs do not act via these traditional mechanisms. The molecular targets for several of these nontraditional AEDs have been defined using cellular electrophysiology and molecular approaches. Here, we describe three of these targets: α2δ, auxiliary subunits of voltage-gated calcium channels through which the gabapentinoids gabapentin and pregabalin exert their anticonvulsant and analgesic actions; SV2A, a ubiquitous synaptic vesicle glycoprotein that may prepare vesicles for fusion and serves as the target for levetiracetam and its analog brivaracetam (which is currently in late-stage clinical development); and Kv7/KCNQ/M potassium channels that mediate the M-current, which acts a brake on repetitive firing and burst generation and serves as the target for the investigational AEDs retigabine and ICA-105665. Functionally, all of the new targets modulate neurotransmitter output at synapses, focusing attention on presynaptic terminals as critical sites of action for AEDs.

Introduction

Since the 1940s, the discovery of antiepileptic drugs (AEDs) has been based on predictive animal models that have allowed the identification of anticonvulsant activity in structurally diverse chemical compounds, some of which were subsequently proven to be effective in the treatment of epilepsy in human clinical trials. Virtually all of the more than 25 distinct chemical entities that have been marketed for the treatment of epilepsy had their origins in this development strategy. Since a consideration of molecular mechanism does not play a role in the development process, AEDs often reach the market with little understanding of their pharmacodynamic effects. However, largely as a result of cellular electrophysiological studies beginning in the 1980s, there has been an evolving understanding of the mechanisms of the clinically used AEDs and the molecular targets on which they act [1]. Until recently, there has been a consensus that the major targets are voltage-gated sodium channels; components of the GABA system including $GABA_A$ receptors, the GAT-1 GABA transporter and GABA transaminase; and voltage-gated calcium channels [2]. In 1993, gabapentin was reported to bind with high affinity to a site in neuronal membranes, which was subsequently demonstrated to represent the $\alpha_2\delta$ protein [3]. Although the implications of this observation were not well appreciated at the time, it marked the beginning of a new era in which AED targets are identified using molecular fishhook and affinity labeling techniques in conjunction with genomics. This has resulted in an expansion in the range of known targets for clinically effective AEDs. It is noteworthy that the traditional process by which new AEDs are discovered, which utilizes animal models that are unbiased with respect to mechanism, enabled the identification of these new targets [4]. Had the initial screens utilized assays based upon the limited range of targets known at the time, the new targets would never have been discovered.

Any brain constituent that plays a role in neuronal excitability mechanisms is a potential AED target. There are thus a large range of prospective targets, including voltage-gated ion channels

(143 subunit genes), ligand-gated ion channels (71 subunit genes), gap junctions (4 connexins genes), G-protein coupled receptors (~600 genes), plasma membrane neurotransmitter transporters (13 genes), neurotransmitter metabolic enzymes, other enzymes affecting ion channel function (such as kinases and phosphatases), and components of the synaptic release machinery (estimated at 50–100 proteins) [5]. A small subset of these potential targets has been validated because of the availability of drugs that act specifically on the targets and the demonstration that these drugs have anticonvulsant activity in animals. This article will focus on several novel targets that have not only been validated in animal models but for which there is evidence that drugs acting on the targets have clinical efficacy in the treatment of epilepsy in humans. These targets are $\alpha_2\delta$, the high-affinity binding site for gabapentin and pregabalin; SV2A, a synaptic vesicle protein to which levetiracetam and brivaracetam bind; and M-type potassium channels, through which the investigational AED retigabine acts.

$\alpha_2\delta$, The Target for Gabapentinoids

Gabapentin was originally developed as an analog of the inhibitory neurotransmitter GABA. GABA, an amino acid, was modified by addition of a cyclohexane ring to its backbone at the 3-position to increase its lipophilicity so as to allow passive blood-brain barrier transport [6]. The concepts that inspired the medicinal chemistry were erroneous as gabapentin does not interact with either $GABA_A$ or $GABA_B$ receptors or other components of GABA systems (such as the GABA transporter or GABA transaminase), and it is transported across the intestinal wall and into the brain by the system-L large neutral amino acid uptake carrier. Pregabalin is similar chemically to gabapentin in that it is an analog of GABA with a bulky aliphatic group (isobutyl) at the 3-position of the GABA backbone. The in vivo anticonvulsant actions and cellular mechanisms of action of gabapentin and pregabalin are similar. Together, they are referred to as “gabapentinoids.”

Studies with [^{3}H]gabapentin demonstrated high-affinity binding in brain, and the binding site was subsequently identified in porcine brain as $\alpha_2\delta$, which is an auxiliary subunit of voltage-gated calcium channels [3, 7]. The protein was detergent solubilized, purified chromatographically, and partially sequenced. A 10 amino acid stretch from the N-terminus was found to be identical with the human $\alpha_2\delta$ sequence deduced by cDNA cloning. In 2001, two additional $\alpha_2\delta$ proteins were identified, designated $\alpha_2\delta$-2 and $\alpha_2\delta$-3 [8•]. The original form, referred to now as $\alpha_2\delta$-1, has the highest binding affinity (K_d, 59 nM). Gabapentin also binds to $\alpha_2\delta$-2 (K_d, 153 nM) but does not bind to $\alpha_2\delta$-3. A subsequently identified $\alpha_2\delta$-4 form, expressed mainly in endocrine and peripheral tissues, also does not bind gabapentin [9]. The $\alpha_2\delta$ proteins are derived from a single gene, the product of which is extensively modified post-translationally, particularly through the cleavage of a signal sequence polypeptide to form separate disulfide-linked α_2 and δ peptides. The $\alpha_2\delta$ proteins are Sanger type I transmembrane proteins (single transmembrane segment with internal carboxy terminus), in which the α_2 subunit is exofacial and the δ subunit is membrane spanning. Mutagenesis and deletion experiments have identified critical regions within the $\alpha_2\delta$ protein that are required for gabapentin binding [10]. In these experiments, it was found that the arginine at position 217 is required for [^{3}H]gabapentin binding [11•]. With this information in hand, a transgenic mouse was created in which the wild-type $\alpha_2\delta$-1 gene was replaced with one encoding a mutated protein in which the arginine at position 217 was replaced by alanine [12••]. These R217A mutant mice had reduced [^{3}H]pregabalin binding in the neocortex, amygdala, entorhinal cortex and hippocampus [13]. Although the mutant mice exhibited pain responses and seizures that were indistinguishable from those of wild-type controls, pregabalin (30 mg/kg) had no analgesic activity although it was analgesic in wild type controls [12••]. The mutant mice still showed an anticonvulsant response to pregabalin in the maximal electroshock test, although this was greatly diminished (M. Vartanian and S. Baron, unpublished). These results indicate that $\alpha_2\delta$-1 binding is required for the analgesic activity of

pregabalin and contributes to its anticonvulsant activity. It seems likely that binding to $\alpha_2\delta$-2 could also play a role in the anticonvulsant activity, thus accounting for the anticonvulsant response in the mutant animals. Indeed, while [^{3}H]gabapentin binding is markedly reduced in R217A mice, it is not eliminated [13]. Areas relevant to seizures, including the hippocampus and amygdala, show 9 to 46% residual binding, which is presumably to $\alpha_2\delta$-2. Although it seems plausible that $\alpha_2\delta$-2 binding could contribute to the anticonvulsant activity of gabapentinoids, definitive confirmation awaits the creation of transgenic mice bearing mutations in $\alpha_2\delta$-2. Additional support for a role of $\alpha_2\delta$ in the anticonvulsant activity of gabapentinoids is provided by structure-activity studies with a variety of compounds related to gabapentin and pregabalin in which there is a strong correlation between $\alpha_2\delta$ binding affinity and anticonvulsant activity [14, 15].

In view of the possibility that $\alpha_2\delta$-2 could contribute to the anticonvulsant actions of gabapentinoids, it is interesting that spontaneous and induced mutations affecting the $\alpha_2\delta$-2 gene have been associated with epilepsy and enhanced seizure susceptibility. Thus, various alleles of the mouse strain ducky, which have disruptions or modifications in the *Cacna2d2* gene that are predicted to alter $\alpha_2\delta$-2 expression or structure, demonstrate absence epilepsy [16, 17]. In addition, mice with targeted disruption of the *Cacna2d2* exhibit a markedly reduced threshold for pentylenetetrazol seizures [18]. As of yet no mutations in $\alpha_2\delta$-1 have been associated with epilepsy in mice, nor are there examples where any of the $\alpha_2\delta$ genes have been linked to human epilepsy.

The exact mechanism whereby gapapentinoid binding to the $\alpha_2\delta$ proteins protects against seizures has not been fully defined. It is plausible that the functional effects of gabapentinoid binding could be related to the role of $\alpha_2\delta$ as a subunit of calcium channels and through effects on calcium flux . However, it cannot be excluded that $\alpha_2\delta$ has other cellular functions that could be relevant to the actions of gabapentinoids [19]. Other studies suggest that the actions of gabapentinoid $\alpha_2\delta$ ligands to reduce neurotransmitter release may not require inhibition of calcium influx, and therefore

may be mediated by an interaction of $\alpha_2\delta$ (or the calcium-channel complex containing $\alpha_2\delta$) with synaptic proteins that are involved in the release or trafficking of synaptic vesicles [20]. Since little is know about these potential interactions, we focus here on $\alpha_2\delta$ as a component of voltage-gated calcium channels.

Studies of calcium flux measured with fluorescent probes in synaptosomes from rat neocortex [21] or human brain tissue obtained at epilepsy surgery [22] have indicated that both gabapentin and pregabalin reduce calcium influx into presynaptic terminals. In addition, gabapentin and pregabalin have been shown to produce subtle but reproducible reductions in the calcium-dependent release of glutamate and other neurotransmitters, including norepinephrine, serotonin and dopamine, from neocortical tissues [23, 24]. However, studies examining the direct actions of gabapentin and pregabalin on calcium channel currents have been equivocal [25]. While some investigators have shown small inhibitory effects on release, others have failed to detect such actions. Similarly, despite the aforementioned studies reporting effects on nerve terminal calcium influx, it has been difficult to reproducibly demonstrate robust acute effects on synaptic transmission. A recent report has proposed that the action of gabapentin is not to directly reduce calcium channel activity, but rather to inhibit trafficking and plasma membrane expression of $\alpha_2\delta$ and the channel complex of which it is a component [26••]. Interestingly, gabapentin must enter neurons to exert this effect on trafficking, which it does via transport by the system-L transporter. It has been speculated that gabapentin displaces an endogenous positive modulatory substance (perhaps L-leucine) that is required for trafficking. Thus, according to this theory, the action of gabapentanoids would depend upon the turnover of calcium channels. The rate of turnover has not been well defined, but it would have to be sufficiently fast to explain the rapid action of gabapentin in animal seizure models, where pretreatment with gabapentin for 60–120 min or less can confer seizure protection [27, 28, 29]. This mechanism represents an entirely novel action for an AED, which contrasts with the usual view that

AEDs directly (or perhaps indirectly in the case of topiramate) modulate channel activity [1]. Clearly, there is much more to be learned about the functional roles of $\alpha_2\delta$ proteins before it will be possible to gain a complete understanding of the mechanism of action of gabapentinoid AEDs.

SV2A, The Target for Levetiracetam and Brivaracetam

SV2A is a membrane glycoprotein present in the synaptic vesicles of neurons and the secretory vesicles of endocrine cells [30]. The protein is believed to be present in virtually all neurons of vertebrates (but not invertebrates). SV2A has a structure that is homologous to bacterial and eukaryotic transporters, with 12 potential transmembrane regions and cytoplasmic N- and C-termini. However, a transport function of SV2A has not been demonstrated. Various hypotheses have been advanced for the function of SV2A including trapping soluble neurotransmitter molecules, diminishing intravesicular osmotic pressure, modifying synaptic vesicle exocytosis by binding to synaptotagmin I, and serving as a scaffold protein to regulate vesicle shape or participate in vesicle trafficking [31]. None of these proposed mechanisms have been verified. Recently, however, Custer et al. [32•] have proposed that SV2A is a positive modulator of synaptic transmission that may act by preparing vesicles for fusion. These authors found a reduction in excitatory responses (EPSP peak amplitude) in hippocampal neuron cultures from mice in which SV2A had been deleted by gene targeting. In addition, the initial synaptic response to trains of stimuli was reduced in neurons from the knockout animals. This effect appeared to be due to a smaller readily releasable pool of vesicles leading to a reduction in initial release probability. Synapses, however, appeared morphologically normal and there was no change in the number of docked vesicles. This led the authors to conclude that SV2A does not influencing docking but rather has a role in facilitating "priming" (the events that render docked vesicles competent for calcium-triggered fusion). It is not apparent how functional alterations in the activity of SV2A and the resultant effects on vesicle dynamics might influence epileptic excitability. However, it is noteworthy that SV2A knockout mice develop severe seizures

and die within the first weeks of life [33]. Heterozygous animal do not show spontaneous seizures, but they do exhibit enhanced seizure susceptibility to convulsant stimuli and an enhanced rate of kindling [34]. Thus, there is a link between SV2A and epilepsy, although the physiological basis remains to be elucidated. Although its functional role is not well understood, SV2A exhibits two well-accepted interactions with xenobiotics. First, botulinum neurotoxin A binds to SV2A (and its B and C isoforms), which allows the toxin to enter neurons [35]. Second, the AED levetiracetam and its analogs bind to SV2A and this interaction seems to mediate the anticonvulsant activity of the drugs. In 1995, [^{3}H]levetiracetam was found to exhibit saturable binding to an abundant site in brain with K_d of 780 nM [36]. Further studies using a higher affinity analogue, UCB 30889, showed that the binding site was SV2A [37]. There was a high correlation between the binding affinities of a series of levetiracetam analogs and their potencies for protection against audiogenic seizures in mice, suggesting that SV2A is the molecular target for anticonvulsant activity. The binding assay has been used to screen a library of 12,000 compounds to identify follow-on AEDs for development. Brivaracetam, the *n*-propyl analog of levetiracetam, was identified with 10-fold greater affinity than levetiracetam for sites in brain labelled with [^{3}H]levetiracetam [38•]. Brivaracetam was more potent than levetiracetam in several animal seizure models and may have an expanded spectrum of activity and greater efficacy in certain models. Like levetiracetam, it demonstrates a large separation between the doses conferring seizure protection and acute neurological impairment although the separation may be somewhat lower. A recent phase II clinical trial in 208 patients demonstrated that brivaracetam produced a dose-dependent reduction in the frequency of seizures in adults with refractory partial seizures [39]. Remarkably, brivaracetam had an adverse event profile indistinguishable from placebo. The results of the clinical trial provide ultimate validation of SV2A as an AED target since binding to this site was the initial screen used to select brivaracetam for further development.

K_v7/KCNQ Potassium Channel, the Target for Retigabine

In 1980, Brown and Adams described the existence of a low threshold depolarization-activated potassium current that they referred to as the M-current, because it was inhibited by the cholinergic agonist muscarine [40•]. The M-current is active in the voltage range for action potential initiation and is therefore of particular importance in regulating the dynamics of the neuronal firing [41]. The M-current turns on slowly following membrane depolarization and it does not inactivate with sustained depolarization (Fig. 1). Although the current was originally described in bullfrog sympathetic neurons, it is also present in brain neurons in the hippocampus, neocortex and elsewhere [42]. In hippocampal pyramidal cells, M-current contributes to the medium duration (~0.1 s) afterhyperpolarization that occurs after a single action potential or bursts of action potentials [43]. The currents underlying the medium afterhyperpolarization cause early spike frequency adaptation, thus dampening the late response to sustained excitatory inputs more strongly than the initial excitatory stimulus. In other words, depolarizing stimuli activate the M-current, which acts as a "brake" on repetitive action potential discharges and burst responses, so that neurons generate a regular, stimulus-graded spike output [44•]. Thus, the M-current is uniquely suited to suppress bursting and epileptiform activity, while permitting maintenance of responses to ordinary excitatory inputs [45•].

The molecular identity of the potassium channels that underlie the M-current was uncovered as a result of studies in the late 1990's on a rare type of idiopathic generalized epilepsy [46, 47]. This form of epilepsy, benign familial neonatal convulsions (BFNC), was found to be due to diverse mutations in novel potassium channel subunits, referred to as KCNQ2 (K_v7.2) and KCNQ3 (K_v7.2), that are homologous to a heart potassium channel subunit KvLQT1 (KCNQ1/K_v7.1), which underlies the cardiac I_{Ks} (delayed rectifier) channel and which, when mutated, is associated with a form of the long QT syndrome. The potassium channel family that includes the cardiac KvLQT1 subunit and its

KCNQ homologs is now referred to as K_v7 [48]. Shortly after the discovery of KCNQ2 and KCNQ3, it was demonstrated that the M-current channel is formed by these subunits as heteromultimers [49]. More recent work has proposed that M-current can also be generated by KCNQ2 and KCNQ3 homomultimers and possibly also heteromultimers with other KCNQ subunits, such as KCNQ5 which to date has not been linked to a human disease [50, 51, 52, 53, 54]. KCNQ2 mRNA is found throughout the rodent central nervous system, with high expression levels in the hippocampus, neocortex and cerebellum [42]. Immunohistochemical studies of the distribution of KCNQ2 protein have generally found that it mirrors the distribution of the mRNA. Areas relevant to epilepsy that have high expression levels include hippocampus, neocortex and amygdala [55]. KCNQ3 is also expressed in hippocampus and neocortex as well as thalamus and cerebellum [56].

The first immunocytochemical studies of KCNQ channels indicated that they have a somatodendritic distribution and are also expressed presynaptically [57]. It is noteworthy that functional M-channels (most likely KCNQ2/KCNQ3 heteromers) are expressed by hippocampal interneurons as well as by principal cells [58]. More recent immunocytochemical studies and patch clamp recordings from different regions of the neuron have provided some surprising results regarding the subcellular localization of the channels. In one recent study the somatic distribution of the channels in hippocampal neurons was confirmed, but the channels were unexpectedly found to be absent from distal dendrites [59] (however see [45]). In other studies, immunohistochemical staining has demonstrated co-localization of KCNQ2 with voltage-dependent sodium channels at nodes of Ranvier [60]. In addition, KCNQ2 and also KCNQ3 immunoreactivity has been detected at axon initial segments [47, 61•]. There is an emerging recognition of the similarities between nodes of Ranvier and axon initial segments, which are sites of action potential initiation and propagation, respectively [62]. The two axonal domains have similar molecular compositions: both are enriched in voltage-dependent sodium channels as well as various adhesion molecules and cytoskeletal proteins

that serve to complex sodium channels, including the adaptor protein ankyrin-G. KCNQ2 and KCNQ3 have an ankyrin-G binding motif similar to that present in sodium channels, which is believed to mediate the interaction and retention of both channel types at the plasmalemma of the node and axon initial segment [61•].

The new information on the subcellular localization of M-channels has allowed a refinement in the understanding of role the channels play in regulating neuronal excitability and how they inhibit the generation of epileptiform discharges. In hippocampal pyramidal neurons, spikes are probably initiated in the axon distal to the initial segment [63]. The initial segment therefore lies between the somatodendritic compartment and the true spike initiation site. M-channels at this pivotal location are well positioned to gate transmission of somatodendritic depolarizations to the site of action potential generation. Because M-channels are slow to activate, rapid depolarizations are relatively unaffected by the axon initial segment M-current. In contrast, more prolonged somatodendritic depolarizations, such as those that occur during epileptiform activity, would be attenuated and less likely to activate action potential firing at the spike initiation zone. In addition, initial segment M-channels may block retrograde spike invasion into the somatodendritic compartments, electrically isolating the axon from the remainder of the neuron. Spikes generate an afterdepolarization (driven by subthreshold persistent sodium current), which trigger further spiking so that bursting occurs. The M-current limits the size and duration of the afterdepolarization preventing its escalation into a somatic spike burst [44].

While M-channels in centeral and peripheral neurons have been localized by immunocytochemistry to nodes [60, 64], they have not yet been demonstrated anatomically on nerve terminals. Nevertheless, it has been known for some time that the selective M-current blocker linopirdine can enhance the depolarization-induced release of various neurotransmitters in brain slices [65] and isolated nerve terminals [66], suggesting a presynaptic localization. Recent evidence

supports the view that presynaptic M-channels serve to inhibit neurotransmitter release [67, 68]. For example, in cultured hippocampal neurons, activation of M-channels reduces the frequency of spontaneous EPSCs [69]. In addition, the frequency of miniature EPSCs (and miniature IPSCs) was also reduced indicating an inhibitory effect on the spontaneous quantal release of glutamate (and also GABA); blockade of M-channels had the opposite effects. Although calcium influx is conventionally believed to be the exclusive trigger for neurotransmitter release, there is evidence that release is modulated by voltage [70]. Therefore, the control of neurotransmitter release by M-current may simply be the result of changes in the terminal membrane potential. Alternatively, there could be more complex interactions between KCNQ channels and the release machinery.

Given the critical role of M-current in regulating the transition to bursting, it is of considerable interest that mutations in *KCNQ2*, and also in a few cases *KCNQ3*, are associated with BFNC, which is characterized by frequent unprovoked seizures beginning in the first days of life and resolving after weeks to months. The mutations reside predominantly in the pore region or the long cytoplasmic C-terminus, and also in the S4 voltage sensor and in the S1-S2 region [47]. In many cases, the mutations cause nearly a complete loss of function of the homomeric expressed channels, but in heteromeric KCNQ2/KCNQ3 channels, which are believed to represent the composition of native channels, there is a 20 to 25% reduction in current. Thus, only a relatively small reduction in current leads to the epileptic phenotype. Complete elimination of the current is lethal, as is the case in mice in which the *Kcnq2* gene was deleted by gene targeting [71]. Heterozygous animals develop normally and lack spontaneous epileptic activity, but are more susceptible to pentylenetetrazol-induced seizures. Similar phenotypes are observed in mice homozygous or heterozygous for the spontaneous *Szt1* mutation, which involves the region of the *Kcnq2* gene that encodes the C-terminus of KCNQ2, as well as other genes [72, 73].

Since reducing M-current enhances neuronal excitability and predisposes to seizures, enhancing M-current might be expected to protect against seizures [41]. Indeed, it has long been

speculated that a neuron-specific potassium channel opener could have anticonvulsant properties [2]. However, experimental support for this concept was first provided when it was shown that a powerful AED retigabine could open potassium channels in cultured neuronal cells [74••]. Retigabine was subsequently found to be a specific opener of M-current channels [75, 76, 77], with effects on KCNQ2–5 and most potent activity on KCNQ3. The main action of retigabine is to shift the current–voltage curve for activation of the channels to the left so that they open at more hyperpolarized membrane potentials (see Fig. 1) [78, 79•]. In addition, retigabine increases the rate at which the channels activate and slows the rate they deactivate. These effects appear to be due to an interaction of retgabine with a key tryptophan residue in the S5 domain of the channel [80••, 81]. This residue is not present in the cardiac KvLQT1 channel, which is resistant to retigabine, thus accounting for a lack of carditoxicity of the drug. A glycine in S6 is also critically involved in channel opening. Wuttke et al. [80••] have proposed that retigabine binds to a hydrophobic pocket in the cytoplasmic domains of S5 and S6, thus stabilizing the open state of the channel (see [47]).

Inhibition of excitatory transmitter output at synapses is a key mode of action of AEDs [1] and indeed modulation of synaptic release is the likely action for the other two targets discussed in this review. Therefore, modulation of presynaptic release by M-channels could represent an important way in which drugs like retigabine protect against seizures. However, is certainly not the only mechanism as – surprisingly – retigabine was able to abolish nonsynaptic bursting in hippocampal neurons [82]. The mechanisms of neuronal synchronization in this situation are obscure, but since synaptic transmission is eliminated, the action of retigabine must relate to its effects on intrinsic neuronal excitability. In rodents, KCNQ2 and KCNQ3 show a gradual maturation so that the characteristic adult axonal distribution of the channels is not present in the early postnatal period [55, 56]. Information on the developmental expression of KCNQ channels in the primate or

human brain is not yet available, which would be useful in predicting the response to KCNQ openers in infants and children.

Validation of KCNQ potassium channels as an AED target has come from several recent clinical trials of retigabine [83]. A phase II dose ranging study in 399 patients with partial seizure (study 205) demonstrated a dose-dependent reduction in seizures [84•]. An as yet unpublished phase III trial with a high dose of retigabine (1200 mg/day) (RESTORE 1) in 305 patients who had failed at least two AEDs showed a highly significant median reduction in seizure frequency and in the number of patients achieving ≥50% decrease in seizure frequency. Results of a second phase III trial with lower doses are expected soon.

The efficacy of retigabine in these various clinical trials strongly supports the concept that positive modulation of M-current (reduction in the threshold for activation) can confer seizure protection. However, retigabine has some known pharmacological actions distinct from its effects on KCNQ channels; most notably, it potentiates $GABA_A$ receptor responses at similar or perhaps slightly higher concentrations than are effective on potassium channels [85]. Therefore, it is of interest that a structurally dissimilar KCNQ activator ICA-27243, which is more selective and does not affect $GABA_A$ receptor responses, also exhibits anticonvulsant activity [86, 87, 88]. This confirms the validity of KCNQ channels as an anticonvulsant target, at least in animal models. An additional orally bioavailable selective KCNQ activator ICA-105665 with activity in chemoconvulsant (pentylenetetrazol), electroshock and kindling models, is entering clinical development (G.C. Rigdon, personal communication).

Conclusions

The discovery of the new AED targets discussed in this review was based on the availability of anticonvulsant molecules identified as having protective activity in animal seizure models. With these molecules in hand, a program of research was initiated that eventually led to the identification

of the novel targets. In the case of $\alpha_2\delta$ and SV2A, affinity ligands were used to localize the distribution of the targets and as fishhooks to snare the target for either automated sequencing or molecular weight determination. Proof that the target is relevant to anticonvulsant activity was ultimately based on the use of genetically engineered mice. In the case of K_v7/KCNQ/M potassium channels, the target was discovered through cellular electrophysiology informed by genetic studies in a rare idiopathic epilepsy syndrome. The knowledge of the targets has been utilized to discover follow-on compounds with improved properties that act by similar mechanisms. Pregabalin, an analog of gabapentin, has already reached the market. Levetiracetam spawned the analog brivaracetam, which was identified specifically as a result of its high affinity for the levetiracetam binding site. There are a host of KCNQ openers in various stages of development, some of which are structurally related to retigabine and others that are not [42, 69]. Clearly, the new set of targets discussed in this review are providing opportunities for the rational discovery of AEDs in a way that was not previously possible when animal models where the only tool.

A common theme for the three molecular targets discussed in this review is that they are are all localized to nerve terminals where they play diverse roles in regulating neurotransmitter release. Actions on presynaptic mechanisms are likely to be the primary way in which AEDs that target $\alpha_2\delta$ and SV2A confer seizure protection. Since K_v7/KCNQ/M potassium channels are also localized presynaptically where they regulate release, AEDs that open these channels also likely act in part through regulation of release (although effects somatodendritic and axon initial segment channels are likely relevant as well). The concept that AEDs act presynaptically to modulate transmitter release contrasts with conventional notions that AEDs confer seizure protection by inhibiting repetitive action potential generation, influencing rhythm generating mechanisms, or enhancing postsynaptic inhibition. The discovery and characterization of the new targets focuses attention on presynaptic mechanisms as a key mode of action for AEDs. In fact, it is likely that sodium channel blocking

AEDs, including phenytoin, carbamazepine and lamotrigine, ultimately confer seizure protection by affecting excitatory neurotransmitter output at synapses [1].

The identification of AEDs that act on new targets has not yet led to the magic bullet that reliably eliminates seizures in drug refractory patients. Nevertheless, the new AEDs have provided improvements in safety, tolerability and pharmacokinetics, and they offer a broader range of options. It remains to be seen whether the agents in the development pipeline that act through the targets discussed in this review, including brivaracetam and retigabine, will offer more substantial benefits. Given the encouraging results of recent clinical trials there is cause for some optimism.

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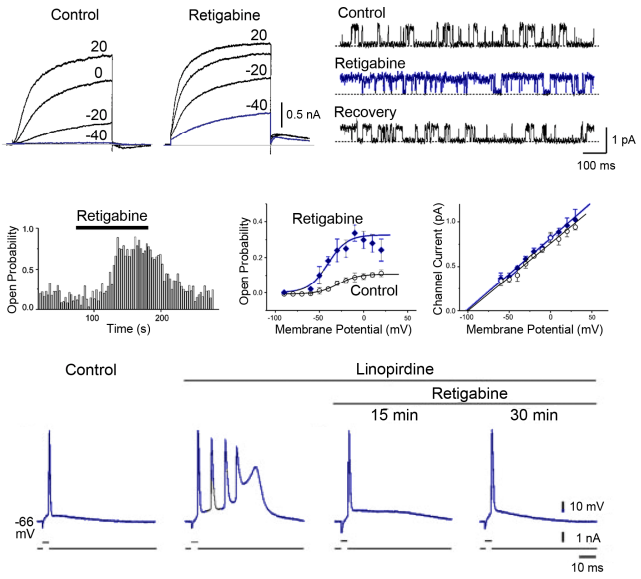

Figure 1. Retigabine activates Kv7/KCNQ channels. *Upper row left*, Whole cell currents from recombinant heteromeric KCNQ2/KCNQ3 channel expressed in CHO cells. Current are activated by 1 sec step depolarization from a holding potential of –70 mV to the indicated potentials. Retigabine (10 μM) shifts the voltage-dependence of activation to hyperpolarized levels. Note the difference between the currents activated at –40 mV in the absence (current nearly at baseline) and presence of retigabine (*blue traces*) (from [78]). *Upper row right*, Single KCNQ2/KCNQ3 channels in a cell-attched patch clamp recording before during and after exposure to 10 μM retigabine. Upward deflections are channel openings. Under control conditions the channel is in the closed configuration most of the time and channel openings are brief (≤ 40 ms) whereas with retigbine the channel is predominantly open with only brief closings (from [79], with permission). *Middle row,* The graph to the left shows the time course of the increase in open channel probability for the channel shown in right upper panel. The middle graph shows the increase in channel open probability as a function of membrane potential. Retigabine shifts the half activation potential from –29 mV to –40 mV. Retigabine does not affect the channel conductance (right graph). *Bottom row,* Intracellular brain slice recording from a rat CA1 pyramidal neuron showing induction of bursting by perfusion with the

specific M-current blocker linopirdine (10 μM) (from [45]). Adding retigabine (10 μM) suppresses the burst response.