

Ion channels in epilepsy

S.M. Mizielinska¹

Neurosciences Institute, Ninewells Hospital, University of Dundee, Dundee DD1 9SY, U.K.

Abstract

Neuronal excitability is determined by the flux of ions through ion channels. Many types of ion channels are expressed in the central nervous system, each responsible for its own aspect of neuronal excitability, from postsynaptic depolarization to action potential generation to neurotransmitter release. These mechanisms are tightly regulated to create a balance between excitation and inhibition. Disruption of this balance is thought to be key in many neurological disorders, including epilepsy syndromes. More and more ion channel mutations are being identified through genetic studies; however, their incidence is still small, suggesting the presence of undiscovered mutations or other causative mechanisms. Understanding wild-type channel function during epileptic activity may also provide vital insights into the remaining idiopathic epilepsies and provide targets for future antiepileptic drugs.

Epilepsy is one of the most common neurological disorders affecting 2% of the world's population. It varies widely in type and severity of seizures and should not be considered as a single disorder. It is currently defined as 'a tendency to have unprovoked recurrent seizures'. Epilepsy can result from brain injury caused by head trauma, stroke or infection, but in 6 out of 10 people seizures have no known cause. Seizures are the result of excessive neuronal firing temporarily disrupting neuronal signalling. This aberrant brain activity is the result of a shift in the balance between excitation and inhibition created by ion channels. Signal transduction in neurons is controlled by electrical signals created by ion flux across the plasma membrane (Figure 1). Positive ion influx upon activation of excitatory receptors (depolarization) competes with negative ion influx through inhibitory receptors (hyperpolarization). Only if the depolarization is sufficient are sodium channels at the axon hillock activated, giving rise to a transient depolarization terminated by delayed potassium channel activation. The depolarization activates nearby sodium channels and is propagated along the axon to presynaptic termini. Here, the depolarization opens calcium channels that stimulate neurotransmitter release, which activates the next neuron in the network, allowing information flow in the brain. Dysfunctions of any of the ion channels involved in this pathway are therefore prime candidates for potential causal agents or possible targets for future antiepileptic drugs.

Excitatory ion channels

iGluRs (ionotropic glutamate receptors) would be expected to play a major role in the hyperexcitable state in epilepsy

syndromes due to their excitatory actions in the CNS (central nervous system). However, unlike other ion channels, no mutations have yet been identified in inheritance studies. Antagonists of iGluRs such as AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors and NMDA (*N*-methyl-D-aspartate) receptors have been shown to have protective effects on *in vitro* models of epilepsy, but also display neurological side effects in humans [1]. AMPA receptor antagonists may have a therapeutic use in the emergency condition status epilepticus where side effects are less important, and also have the advantage over current drugs that they do not impair cardiovascular function and may protect against glutamate-induced excitotoxicity. Two new antiepileptic drugs, topiramate and felbamate, do have effects on glutamatergic excitatory transmission, but it is not fully confirmed whether this is their only mechanism of action.

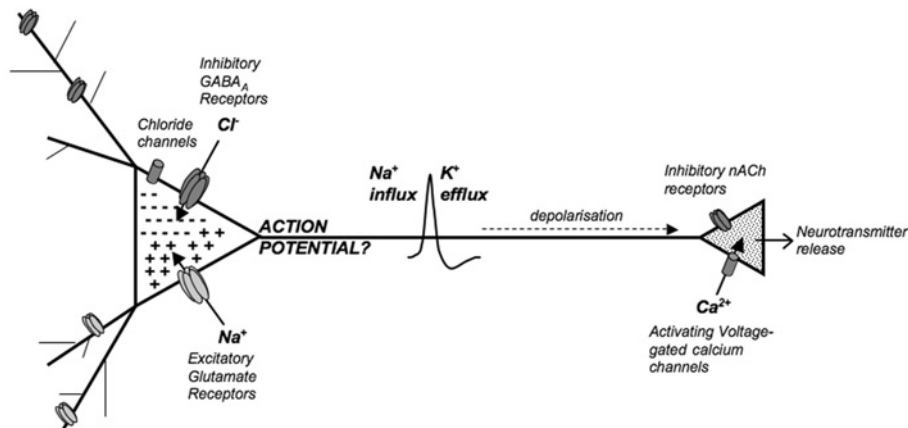
Inhibitory ion channels

A reduction in GABA (γ -aminobutyric acid) inhibition has long been implicated in epilepsy, with GABA_AR [GABA_A (GABA type A) receptor] antagonists promoting epileptic seizures and agonists exhibiting anticonvulsant activity. Enhancement of GABA_A inhibitory transmission is the primary mechanism of benzodiazepines and phenobarbital and is a mechanistic approach to the development of novel antiepileptic drugs. These include tiagabine, which blocks GABA re-uptake, and vigabatrin, which blocks GABA metabolism. A number of GABA_AR mutations associated with epilepsy have been discovered in the α ₁-, γ ₂- and δ -subunit genes (*GABRA1*, *GABRG2* and *GABRD*). Each mutation is reported to cause a reduction in GABAergic inhibition and thus hyperexcitability of neurons [2]. The R43Q mutation found in the γ ₂-subunit of patients with childhood absence epilepsy or febrile seizures causes retention of receptors in the endoplasmic reticulum recently found to be due to the perturbation of the β - γ interface. The γ ₂(K289M) GEFS (generalized epilepsy with febrile seizures)

Key words: action potential, antiepileptic drug, epilepsy mutation, ion channel, neuronal excitability, trafficking.

Abbreviations used: ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; AMPA, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNS, central nervous system; GABA, γ -aminobutyric acid; GABA_A, GABA type A; GABA_AR, GABA_A receptor; GEFS, generalized epilepsy with febrile seizures; iGluR, ionotropic glutamate receptor; nAChR, nicotinic acetylcholine receptor.

¹email s.m.mizielinska@dundee.ac.uk

Figure 1 | The role of ion channels in neuronal signalling anticonvulsant activity

mutation exhibits changes in physiology consistent with its position in the extracellular loop implicated in channel gating. The α_1 (A322D) mutation associated with juvenile myoclonic epilepsy has been reported to be retained in the endoplasmic reticulum where it is degraded, like the γ_2 (R43Q) mutation, decreasing the cell-surface expression and GABA potency. Mutations in the δ -subunit are associated with GEFS (E177A) or juvenile myoclonic epilepsy (R220C) and are both located within the extracellular N-terminal domain, displaying reduced current amplitudes and cell-surface expression. In addition to the genetic predisposition in epilepsy, a reduction in GABA_AR function is also induced by epileptic activity as a result of receptor endocytosis [3,4]. Indeed, receptors containing mutations in the γ -subunit but not the α -subunit have been reported to exhibit impaired trafficking or accelerated endocytosis at raised temperatures that may be relevant to febrile seizures [5].

The gene encoding the voltage-gated chloride channel CLC-2 (*CLCN2*) was first identified in a susceptibility locus for common idiopathic epilepsies. Three mutations have now been identified in the gene: two truncations causing loss of function and one single-residue substitution resulting in a change in voltage dependence [6]. Disruption of CLC-2 channel function could perturb the maintenance of the transmembrane chloride gradient essential for GABA_AR inhibition.

Voltage-gated ion channels involved in action potentials

Sodium channel inhibition is already the target of antiepileptic drugs on the market, including carbamazepine, phenytoin and lamotrigine, and may also be the mechanism for other classic drugs. This is an obvious target for antiepileptic treatment as sodium channels are responsible for the excitatory depolarization in the rising phase of an action potential and enhanced channel activity promotes neuronal hyperexcitability. Voltage-gated sodium channels consist of a pore-forming α -subunit and two small accessory β -subunits that modulate channel kinetics. The β (C121W) missense

mutation in the *SCN1B* gene is primarily associated with GEFS plus, which shifts the voltage dependence and reduces rundown during high-frequency channel activity [7,8]. Other mutations have been found to be associated with GEFS plus in the α -subunit genes *SCN1A* and *SCN2A*, both of which result in an enhanced sodium current [9]. However, more than 100 of the mutations found in the pore-forming subunit gene *SCN1A* are *de novo* mutations (not found in either parent) and have been reported to be associated with more severe epileptic conditions, mutations confusingly resulting in both increased and decreased function of the channel.

Potassium channels are essential in the repolarization and hyperpolarizing overshoot in action potentials. Mutations in *KCNQ2* and *KCNQ3*, the pore-forming subunits for the M-current known to regulate action potential initiation, are the substrates for benign familial neonatal convulsions [10–12]. More than 50 mutations have been identified, which result in up to a 60% reduction (or complete ablation) in current amplitude or disrupt the residues required for calmodulin binding and regulation [13]. The rare syndrome, episodic ataxia type 1, has been closely associated with mutations in the *KCNA1* gene, which have been shown to result in up to a 95% reduction in current amplitudes and dominant-negative effects on wild-type subunits [8]. The KCNQ family is an important new target for antiepileptic drugs, such as retigabine, identified using an *in vivo* screening approach for compounds with potent activity in animal models of epileptic seizures distinct from known anticonvulsants, and has relatively mild side effects compared with other drugs.

Ion channels involved in neurotransmitter release

Calcium channels are key mediators of calcium entry into neurons in response to membrane depolarization, mediating a number of essential functions including the release of neurotransmitters and the regulation of neuronal excitability. Missense mutations have been identified in genes encoding low-voltage-activated T-type channels ($Ca_v3.2$,

CACNA1H; $Ca_v3.1$, *CACNA1G*), high-voltage-activated P/Q-type channels ($Ca_v2.1$, *CACNA1A*) and the regulatory auxiliary β_4 -subunit (*CACNB4*). These have been mainly associated with absence epilepsies, and the anti-absence effect of ethosuximide is due to the inhibition of thalamic T-type channels [14]. Some of the T- and P/Q-type channel mutants display a hyperpolarizing shift in the voltage dependence of activation in transient expression systems resulting in increased channel activity, and thus increase their subsequent activation of neurotransmitter release, whether solely excitatory (T-type) or both excitatory and inhibitory (P/Q-type) neurotransmission. However, most of the mutations do not significantly affect the biophysical characteristics of the channel; a few have been shown to affect alternative splicing [15]. The other auxiliary subunits ($\alpha_2\delta_2$ and γ) have also been implicated in epilepsy by mutations in their genes (*CACNA2D2* and *CACNG2/4*) associated with mouse strains prone to seizures, although no mutations have yet been identified in these genes in humans. $\alpha_2\delta_2$ ligands such as gabapentin and pregabalin are an evolving drug class that binds to hyperexcited calcium channels characterized by their reduction of excessive neurotransmitter release [16].

Mutations in the nAChR (nicotinic acetylcholine receptor) subunits are associated with the rare epilepsy syndrome ADNFLE (autosomal dominant nocturnal frontal lobe epilepsy), characterized by partial seizures predominantly during sleep. Identified missense mutations in the transmembrane regions of α_4 - (*CHRNA4*) and β_2 - (*CHRN2*) subunits, two of the common nAChR subunits in the CNS, appear to reduce the calcium potentiation of channels [17]. A mutation identified in the neuromuscular junction nAChR α_2 -subunit is also associated with ADNFLE, which increases the receptor's sensitivity to acetylcholine [18]. It has been proposed that reductions in channel activity may decrease inhibitory neurotransmitter release or enhance excitatory neurotransmitter release by relieving presynaptic inhibition. Although this mechanism is still not fully understood, the involvement of nAChRs in ADNFLE is supported by the good response of patients to carbamazepine, an inhibitor of nAChRs.

Concluding remarks

Ion channels are prime candidates for potential causal agents or targets for future antiepileptic drugs, due to their individual roles in neuronal excitability. The understanding of the structure–function relationships of ion channels and their role in hyperexcitability has been greatly enhanced by the analysis of mutations identified by genetic studies in familial epilepsies. It must be noted, however, that epilepsy mutations have only been identified in a few per cent of cases worldwide. Moreover, the presence of an epilepsy mutation often leads to increased susceptibility rather than an epilepsy phenotype.

Many epilepsy syndromes are also complex and polygenic, involving environmental factors and *de novo* mutations. Some of these mutations affect the trafficking of the channels to the cell surface, but as seen with the GABA_ARs, wild-type channel trafficking can also be affected. Auxiliary proteins that regulate ion channel function and trafficking may also contribute; mutations have recently been identified in *LGII*, associated with KCNA potassium channels, and *EFHC1*, reported to increase R-type calcium currents [17]. Further studies characterizing ion channel mutations and trafficking during *in vitro* models of epilepsy, in combination with elucidating the mechanisms of action of effective antiepileptic drugs are needed to develop targets with specific actions and therefore fewer neurological or peripheral side effects.

S.M.M. is supported by a BBSRC (Biotechnology and Biological Sciences Research Council) CASE (Co-operative Awards in Science and Engineering) studentship with Merck, Sharpe and Dohme.

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Received 24 July 2007
doi:10.1042/BST0351077