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Voltage Gated Ion Channels as Therapeutic Target for Drug Discovery

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Abstract

Epilepsy is a disorder of central nervous system characterized by spontaneous seizures. The seizures result from neuronal hyperexcitability and hypersynchronus neuronal firing. Inspite of number of drugs available for the treatment of epilepsy approximately 30% patients continue to have seizure episodes and the rest suffer from drug related side effects. This underlines the need for development of safer and effective antiepileptic drugs. Voltage gated ion channel (VGIC) governs the excitability of neurons and perform various function ranging from initiation of action potential, synaptic transmission, neurotransmitter release, termination of action potential to stabilization of neuronal membrane around resting potential. Channelopathies of these ion channels is known to be associated with epileptic seizures. Various drugs either available in the market or in the experimental use have been known to act on these VGICs. This review focuses on the physiological role and selective targeting of these ion channels (sodium, potassium, calcium and Hyperpolarization activated cyclic nucleotide gated (HCN) channels) for design and development of potent and safer efficacious antiepileptic drugs.

Keywords: Epilepsy, Voltage gated ion channels, Channelopathies, Antiepileptic drugs

Introduction

Epilepsy is a collection of complex disorders of the brain, which is characterized by onset of spontaneous convulsant and non-convulsant seizures. These seizures result from neuronal hyperexcitability and hypersynchronus neuronal firing. Around 50 million people in the world are suffering from epilepsy [1]. Our understanding of the pathophysiology of the disease has advanced in last 30 years and eleven newer drugs have been introduced since 1978. Still one third of patients continue to have seizures and the remaining suffer from unacceptable medicinal side effects. Therefore, there is substantial need to design safer and more effective antiepileptic drugs (AEDs)[2,3].

Action potential or neuronal excitability encodes and processes information within nervous system and regulates various physiological processes [4]. This action potential is mediated with the help of various voltage gated ion channels (VGIC) such as; (i) voltage gated sodium channels (VGSC), which initiate action potential, (ii) voltage gated calcium channels (VGCC), which initiate processes like muscle contraction, synaptic transmission, neurotransmitter and hormonal release in response to membrane depolarization, (iii) Voltage gated potassium channels (VGKC), which terminate action potential and return the membrane potential to its resting value and (iv) Hyperpolarization activated cyclic nucleotide gated (HCN) channels, which stabilize the neuronal membrane around resting potential[5,6]. Therefore, voltage gated ion channels keep the normal cell excitability under control by regulating their membrane potential, synaptic transmission and firing rate. Mutation causing functional changes in these ion channels can explain the cause of hyperexcitability of neurons leading to epileptic seizures [7]. Many currently available AEDs act on these ion channels, but there are some other ion channels that can be targeted for development of newer AEDs [7]. As ion channels are expressed very specifically and perform very peculiar functions, it is possible to target specific ion channels for design and development of newer AEDs with lesser side effects. This review will focus on targeting voltage gated ion channels for designing of newer and safer AEDs.

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Voltage Gated Sodium Channels (VGSCs)

VGSCs are membrane proteins that form ion channels allowing the conduction of Na+ ion through it and they play a fundamental role in rising phase of the action potential. It consists of a large α-subunit (260 kDa) of six transmembrane domain associated with two auxiliary β-subunits [8]. These β subunits are single transmembrane segment of 35 kDa (β1–β4) that modulate the properties of the α-subunit and are implicated in its sub cellular targeting [8]. The α-subunit has four domains designated as I-IV, each containing six α-helical transmembrane segments, labelled as S1-S6 (Fig.1).

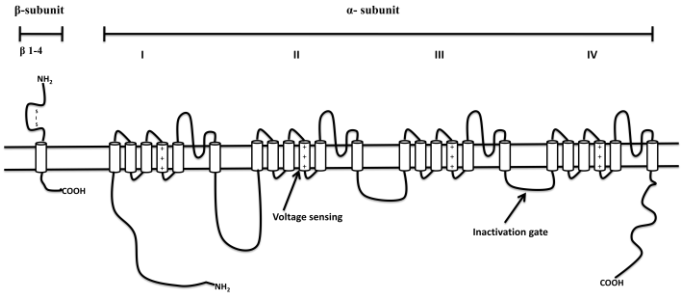

Figure 1. Structure of voltage gated sodium channels[9]

The S4 segment of each of the domain acts as a voltage sensor due to presence of repeated motif of positively charged amino acids [8,9]. Membrane depolarization induces outward movement and rotation of S4 segment in each voltage sensing module, which is physically coupled to opening of the pore by the S4-S5 linker [10]. The loop between each S5 and S6 segment is responsible for its ion selectivity. The four S6 segments which form cytoplasmic end of the pore also provide binding sites for various classes of therapeutically important antiepileptic drugs (AEDs), local anaesthetics and anti-arrhythmic drugs[8,9]. The intracellular loop between domain III and IV forms the fast inactivation gate that occludes the cytoplasmic end of the pore when the channel inactivates [8,9]. The C-terminal contains binding site for interacting proteins and is necessary for some of the fast inactivation properties [8,9]. VGSCs remain closed at resting membrane potentials which is the characteristic of inactive neurons. Membrane depolarization leads to opening of these channels resulting in inward movement of sodium ions. Once the depolarization phase is over; these channels get converted into inactivated state i.e. fast inactivation. Sodium channels in the brain can cycle through these states in milliseconds, allowing them to sustain rapid trains of action potentials, which are essential for brain function. Incomplete inactivation of Na+ current in many neurons results in slow inactivating current also known as persistent Na+ current (INaP). This is mainly responsible for important effects on the neuronal functions such as increase in synaptic potential, increased firing frequency at subthresold potentials and maintaining the depolarized plateau potential for a longer time. Neurological disorders such as epilepsy and neuropathic pain are associated with higher amplitude of INaP as compared to normal physiological conditions in the body. Therefore, small modification in INaP can significantly alter neuronal properties and alleviate the disorders [9].

Nine α-subunit subtypes have been cloned and functionally expressed. These sub types are designated as Nav1.1 – Nav1.9 [8]. Out of these, Nav 1.1, Nav 1.2, Nav 1.3 and Nav 1.6 are found predominantly in the central nervous system. Nav 1.1 and Nav 1.2 are isoform (predominant in the cerebellum, thalamus, rostral brain, globus pallidus and hippocampus) and Nav1.6 (predominant in the somatodendritic regions of output neurons of the cerebellum, cortex and hippocampus) mediate the fast transient Na+ current and generate action potential. These channels also mediate the constant, resurgent or late Na+ current, which may play significant role in epilepsy [9, 11-13].

Altered concentration of protein and m-RNA for α-subunits Nav1.1, Nav1.2, Nav1.3, Nav1.6 and also for β-subunits have been reported in animal models of acquired and inherited epilepsy as well as in brain tissues of humans suffering from epilepsy. This suggests that they play an important role in the process of epileptogenesis or in the maintenance of the epileptic state [9,11,14-17]. It was later supported by the fact that

the epileptic syndromes like benign neonatal-infantile familial seizures, simple febrile seizures, generalised epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy of infancy (SMEI) also known as Dravet's syndrome are linked to mutations in sodium channels[18-21].

Approximately two dozen drugs are available in the market for the treatment of epilepsy and most of them are sodium channel blocker (e.g. Phenytoin, Carbamazepine, Valproic acid, Lamotrigine, Rufinamide). Despite of this fact, 30% patients continue to have seizures even during the therapy, while others continue to suffer from medication induced side effects. Thus, there is an urgent need to continue the search for new VGSC blockers having better efficacy and less side effects [22]. The following criteria can be taken in consideration while designing newer sodium channel blocker for epilepsy treatment. (i) Design and development of higher level of voltage dependent and use dependent blockers of sodium channels which will bind at inactivated state. This will suppress high frequency, repetitive, action potential firing so that interference with normal physiological function can be minimized. (ii) Various sodium channels like Nav1.1, Nav1.2 or Nav1.6 have different patterns of expression in different brain regions and are associated with pathophysiology of specific disorder including epilepsy. Thus selective blocking of specific channel subtype would serve the purpose of increasing the therapeutic outcome and reducing the side effects. (iii) To develop Na+ channel blocker AEDs with multiple mechanisms of action, e.g. various marketed AEDs that are known to block Na+ channels but also have prominent effect on other targets (Felbamate, a positive modulator of GABAA receptor, blocker of NMDA receptor and voltage gated sodium channels). Such multiple mechanisms may yield unique clinical efficacies of each of these drugs. These characteristics will provide newer Na+ blocking AEDs with improved efficacy and reduced side effects [11].

Voltage Gated Potassium Channels (VGKCs)

Potassium (K+) channels are the most widely distributed types of ion channels forming pores that span the cell membrane. In mammals their subunits are encoded by approximately 80 Potassium channel (KCN) genes, making them the most diverse of all ion channel families. They are expressed throughout the body and have various physiological functions. These channels are grouped into different families depending on the number of transmembrane (TM) spanning regions of each subunit as well as their physiological and pharmacological functions. It consist of (i) 2 TM, inward rectifying potassium channels (ii) 4 TM, two pore potassium channels (iii) 6 or 7 TM, calcium activated potassium channels & (iv) 6 TM, voltage gated potassium channels. In this review, we will focus on the largest gene family within the K+ channels i.e. voltage gated potassium channels (Kv). In human beings, these channels are encoded by 40 genes and are divided into 12 subfamilies (Kv 1- Kv 12)[23,24].

Mammalian voltage gated potassium channels consist of four α-subunits, each containing 6-TM (S1- S6) region forming a single pore (Fig.2).

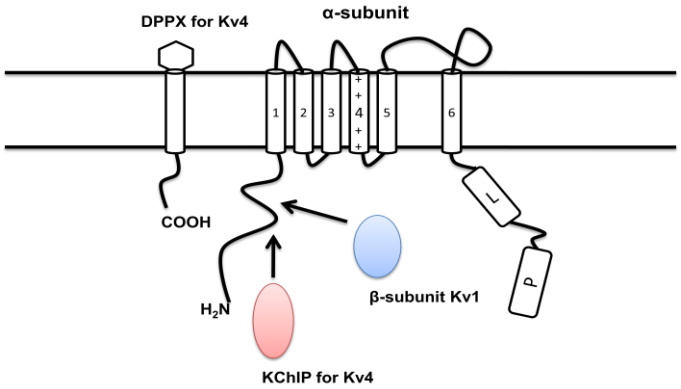

Figure 2 Structure of voltage gated potassium channels [25,27].

These potassium channels have a distinctive P loop motif (TVGYG; threonine, valine, glycine, tyrosine, glycine) between S5-S6 which is responsible for potassium selective permeability, while S4 domain act as voltage sensing domain. The N terminus of the α-subunits (Kv1)

is associated with auxiliary β-subunits (β1-β3) which regulate channel inactivation as well as its expression [24]. N-terminus of the Kv4 α-subunits is associated potassium channel interacting proteins (KChIPs), calcium binding proteins. Kv4 channels are also associated with a single trans-membrane spanning protein with large extracellular domain known as dipeptidyl aminopeptidase X (DPPX)[25]. The KChIPs have the property to alter the Kv4 gating whereas, DPPX is linked with facilitation of α-subunit (Kv4) trafficking to the membrane and increasing the rate of inactivation. The C-terminus of Kv2.1 α-subunit may be associated with localization domain (required for correction localization of Kv2.1 channels in cells)[26] or postsynaptic density protein (PSD-95; a synapse associated protein possessing PDZ domains) in Kv1 and 4.2[27]. The PSD-95 may affect cell surface levels, current amplitude, activation or inactivation kinetics of ion channels[27]. The K+ concentration gradient across the membrane is maintained due to efflux of potassium ion on opening of the channel. This gradient determines the repolarization phase that follows the depolarization of cell membrane. Kv also plays an important role in Ca2+ signalling, volume regulation, secretion, proliferation and migration in both excitable and non-excitable cells[29].

Kv channel's subunits are expressed throughout the central and peripheral nervous system in both neurons and glial cells. In neurons, Kv1 (KCNA) channels are predominantly expressed in axons and nerve terminals, Kv2 (KCNB) and Kv4 (KCND) channels are expressed in the somato-dendritic domain, Kv3 (KCNC) channel are expressed either in dendritic or in axonal domain, depending on splice variant or the neuronal cell type. Kv7 (KCNQ) are predominantly expressed in initial segment of axons, nodes of Ranvier and some evidence of expression in somatodendritic region [29, 30]. Furthermore, Kv5, Kv6, Kv8 and Kv9 are unique within the Kv channel family as they are functional only when they co-assemble with Kv2 subunits, which modifies their function [31].

A-type of K+ current is responsible for repolarization of action potential and is mediated by Kv1, Kv3 & Kv4. Reduced expression and functionality of Kv1.1 and Kvβ assembles with Kv1 channels have been reported in the experimental model of temporal lobe epilepsy (TLE)[32]. Kv7 channels mediate M-current which is important regulator of the medium after- hyperpolarization of action potential. Mutation causing loss of function of Kv7 (Kv7.2 and Kv7.3) channels have been associated with benign familial neonatal seizures (BFNS), peripheral neuronal hyperexcitability (PNH), and epileptic encephalopathy [29,33]. 4-aminopyridine (4-AP; a blocker of Kv1, Kv3 and Kv4 channels), variety of peptide toxins from snake, sea anemones and scorpions selectively block Kv channels causing seizure in rodent models [11, 34-36].

Considering the importance of Kv7.2/7.3 in epilepsy, efforts have been directed towards developing therapeutic agents that target these channels [24]. Retigabine is first-in-class antiepileptic that reduces neuronal excitability by enhancing the activity of Kv7 channels. It causes stabilization of neuronal Kv channels by increasing outward K+ current in the open position leading to hyperpolarization of the sub-threshold membrane potential. This opposes the depolarizing Na+ current required for multiple action potential generation in hyper excitable condition[37]. It selectively targets 7.2-7.5 channels but does not activate 7.1 channels, thus reduces cardiac side effects [38]. It has been approved as adjunctive therapy for adults with partial seizure by European Medicines Agency and the US Food and Drug Administration [29]. Several other classes of compounds like phenylacrylamides, benzisoxazoles and benzamides have been shown to act as KCNQ2–5 (M-current) channel openers. Some of these protect against seizures in animal models and are more specific for KCNQ channels than - Retigabine, ensuring that opening of KCNQ channels is a valid molecular target for AEDs [11,39]. The Kv1, Kv3, and Kv4 channels that underlie A-type currents are also attractive AED targets for AED development [11].

Voltage gated calcium channels (VGCCs)

Voltage gated Calcium channels (VGCCs) are a family of ion channels that mediate Ca2+ entry into living cells upon membrane depolarization, which will eventually lead to various physiological processes such as muscle contraction, secretion of hormone, neurotransmitter release and regulation of gene expression. Therefore, the healthy status of cell depends on the regulation of calcium level in the cell [40-43]. Structurally, VGCCs are hetero multimeric assemblies composed of a central α subunit and various auxiliary subunits (β1-4, α2-δ1-4 and γ1-8) (Fig. 3).

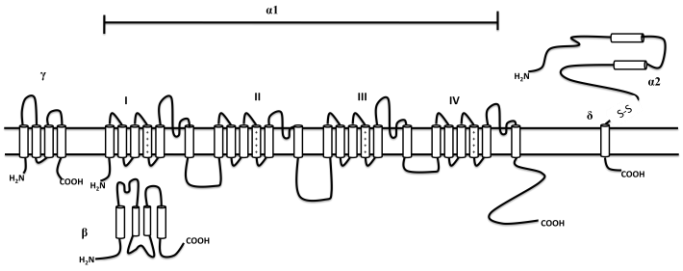

Figure 3. Structure of Voltage gated Calcium Channels [48].

The main component of the VGCC is α subunit (approximately 190-250kDa), which is structurally similar to VGSC α-subunit (four repeated domain (I-IV) each of which is 6 TM spanning segments). Similar to its sodium and potassium conducting counterparts, its S4 membrane spanning domain act as voltage sensor and the pore is formed by re-entrant loop between the S5 and S6 segments[44-46]. As with that of voltage gated sodium channels, the auxiliary subunits (β, α2-δ and γ) are not essential for the formation of functional ion conducting pores but are required for conferring correct gating kinetics and cell surface expression of the ion channels [46]. β-subunit (β1-4) in VGCCs (53-65kDa) are protein found in the intracellular compartment, which induces large changes to the voltage dependence of activation and the rate of inactivation. α2-δ subunit (α2-δ1-4; 123-129kDa) are the dimmers linked to the α1 subunit, of which α2 subunit is the extracellular subunit that interacts mostly with α1 subunit. A single transmembrane region with a short intracellular portion of δ subunit anchors the protein in the plasma membrane. Furthermore, several γ subunit (γ1-5; 25-36 kDa) have been indentified but their expression and functional status are relatively unknown [44-46].

Ten different Cav-α1 (voltage gated calcium channels) have so far been identified through homology screening and pharmacological properties. This has led to classification of the different Ca2+ channel types as (i) High voltage activated (HVA) Ca2+ channels that are activated at stronger depolarizing potential (approximately -50 to 30 mV). They display higher single channel conductance and prolonged channel opening compared to low voltage activated (LVA) calcium channels. HVA activated Ca2+ channels are further divided into L (Long lasting) type or dihydropyridines (DHP – like Amlodipine) sensitive type of calcium channels (Cav1.1-Cav1.4) and non L type or less DHP sensitive type (Cav2.1-P/Q, Cav2.2-N, Cav2.3-R) of calcium channels[45-48]. The non L type of calcium channels (N, P/Q and R) are further classified as; N-type (N-new current) of calcium channels, which are activated at more negative potential than L-type but more positive potential than T-type (hence new current-N type). These channels show more rapid inactivation than L but slower inactivation that the T-type[49]. These channels are blocked by the snail peptide ω-conotoxin GVIA, which is the crucial distinguishing factor for identification of N-type channels [50]. P-type channels were first recorded in purkinje neuron and could be blocked by spider toxin ω-agatoxin IVA. The Q-type was first found in cerebellar granule neurons and has much lower affinity for the toxin. Finally, R-type of calcium channels, which are found in cerebellar granule neurons and got their name from the fact that they are resistant to the subunit specific organic and peptide Ca2+ channel blocker[46], (ii) Low voltage activated (LVA; Cav3.1-3.3) calcium channels also known as T (transient/tiny) type of calcium channels. These types of calcium channels are found in neuronal cell bodies and dendrites and are activated at more negative membrane potential

(approximately -70 mV). They exhibit fast inactivation and small single channel conductance [47, 48, 51].

VGCCs play an important role in pathogenesis of epilepsy because of their electrophysiological properties and their cellular distribution [23, 47]. Reticular thalamic nucleus (RTN) and thalamic relay cells are two import regions of thalamo-cortical circuit involved in absence seizure. RTN showed presence of Cav3.2, Cav3.3, Cav2.1 and Cav2.3 type of VGCCs whereas, thalamic relay neurons showed presence of Cav3.1 and 2.1 types of calcium channels [43, 52, 53]. Animal models of absence epilepsy such as the one described by Ernst et al., (2009) and others like Genetic Absence Epilepsy Rats from Strasbourg (GEARS)[54] and Wistar Albino Glaxo rats from Rijswijk (WAG/Rij)[55] revealed that mutation in LVA-type calcium channels is associated with enhancement in its activity. Same models showed that mutation in HVA type of calcium channels is associated with decrease in their function. In human beings, childhood absence epilepsy has been reported to be associated with a missense mutation of Cav3.2 leading to enhancement in its function [56].

LVA T-type of calcium channels have been associated with epileptiform spike and wave discharge in absence seizure. Thus, drug blocking T-type of calcium channels may serve as good target of drug against absence seizure e.g Ethosuximide (ETX), which reduces the hyperexcitability of thalamocortical neurons associated with absence seizure [43,57]. Newer AED like Gabapentin, which was designed as CNS penetrating GABA analogue but later it was found that is has no effect on GABA receptors, enzyme or transporter. It was later proved that both Gabapentin and Pregabalin bind specifically to α2-δ1 and α2-δ2 but not to α2-δ3 or α2-δ4 thereby inhibiting depolarization induced calcium influx into the cell [58,59]. These drugs have been proved to be effective against wide range of animal models and have been approved as add on therapy. Moreover, clinically effective drugs like Lamotrigine (LTG) and Topiramate (TPM) have also shown some activity against VGCCs [60].

As thalamo-cortical and RTN neurons have the property to switch between the tonic (vigilant mode), intermediate and burst firing mode (pathological factor activated in absence seizure due to LVA calcium channel), activation of tonic mode (through activation of HVA channels) can serve as an alternative therapy to block the LVA T-type calcium channels in absence seizures. Genetic animal models have pointed that decrease in function of HVA type of calcium channels is associated with absence seizure. The control of absence seizure can be obtained with activation of HVA calcium channels. This was confirmed by spike wave discharge (SWD) suppressive effect of L-type of Ca2+ channel agonist (BayK-86444)[43]. It was observed that SWD enhancing effect of L-type of calcium channel antagonist increased the seizure susceptibility of mice in a similar manner as mice deficient in Ca2.3 Ca2+ channels [61].This confirmed the role of HVA calcium channels in pathogenesis of seizure activity [43].

Hyperpolarization activated cyclic nucleotide gated (HCN) channel

Hyperpolarization activated cyclic nucleotide gated (HCN) channels belong to super-family of voltage gated pore loop channels. The HCN channels were first identified in heart as pacemaker channels which are now understood to be an important modulator of neuronal excitability. These channels allow the passage of depolarizing current i.e. hyperpolarization activated current (Ih) or the funny current (If), which play a key role in controlling the rhythmic activity in cardiac pacemaker cells and spontaneously firing neurons. However HCN channels possess biophysical properties that make them virtually unique as compared to other voltage gated ion channels [6, 62,63]. Although, these channels are structurally similar to K+ channels, under physiological condition mainly at resting membrane potential, these channels are selective for Na+ ions rather than K+ ions. Therefore, these channels allow inward passage of Na+ ions and

eventually depolarizing neuronal membrane potential. Similarly, the voltage dependent activation of HCN channels is strange as compared to most other ion channels. The activation of HCN channel increases with hyperpolarisation unlike the other voltage gated ion channels which are activated via depolarization. Therefore, neuronal hyperpolarization tends to activate the HCN channels and depolarization turns them off them. These channels remain constitutively activated around resting membrane potential. These channels open remarkably slowly with activation (several times slower than most of other ion channels). Finally, HCN channels are partly gated by intracellular levels of cyclic adenosine monophosphate (c-AMP). Thus, allowing channel activity to be modulated by both voltage and intracellular second messengers system. The biophysical property of these channels results in stabilizing the neurons at its resting potential minimizing the influence of synaptic inputs. When neurons become depolarized by synaptic input, the tonic depolarizing Na+ current mediated by HCN channel is turned off. Hyperpolarizing input will activate the HCN channels allowing more Na+ ion influx, thus returning cell membrane to the resting membrane potential. Thus, Ih displays as inherent negative feedback property that imparts a stabilizing effect on neuronal excitability. The HCN channels can stabilize the neuronal cell membrane in case of both excitation as well as inhibition. This is also regulated by various other factors like membrane phosophatidylinositol-4, 5-bisphosphate (PIP2), intracellular and extracellular proton concentration and concentration of extracellular chloride ions. These factors are essential for controlling the cell surface expression of ion channels, their functional properties and targeting to defined cellular compartment [6, 63-65].

The core unit of HCN channels has similar structural design to that of many other pore loop channels, consisting of four subunits that are arranged around the centrally located pore. Each HCN channel consists of two major structural modules, the transmembrane core and cytosolic C-terminal domain (Fig. 4). The trans-membrane core harbours the gating machinery and the ion conducting pore. The cytosolic C-terminal domain confers modulation by cyclic nucleotides. Both of these modules co-operate each other during channel activation [65]. Each trans-membrane channel core consists of six helical segments (S1-S6) and an ion conducting pore loop between S5 and S6. It also has highly conserved asparagine residue in the extracellular loop between S5 and the pore loop which is glycosylated. This post-transitional channel modification is crucial for normal cell surface expression [64-66]. The voltage sensor of HCN channels is formed by a charged S4 helix [65,67,68]. HCN channels contain glycine-tyrosine-glycine motif which are selective filter for K+ ion. These channels also conduct Na+ and K+ with permeability ratios of about 1:4 however; at physiological condition i.e. at resting membrane potential, HCN channel preferentially conduct Na+ ions. These channels are blocked by Caesium (Cs+) [65,69]. The cytosolic C-terminal domain of HCN channels contains an 80 amino acid long C-linker region followed by cyclic nucleotide biding domain of 120 amino acids. Binding of cAMP to the Cyclic Nucleotide Binding Domain (CNBD) speeds up channel opening and shifts the voltage dependence of activation to more positive voltage. Thus, at the given voltage the current flowing through HCN channel is larger in presence of c-AMP than in its absence [69].

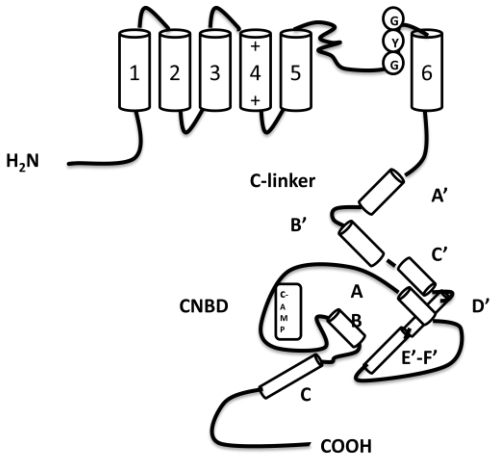

Figure 4. Structure Hyperpolarization activated cyclic nucleotide gated (HCN) channel [64].

HCN channels are encoded by four separate genes (HCN1-4). Ion channels encoded by each of these isoform have differing biophysical properties such as speed of gating and sensitivity to c-AMP. These channels are differentially distributed throughout the brain. HCN1 and HCN2 are the main isoforms in the brain. HCN1 channels are predominantly found in cerebral cortex, hippocampus and brainstem and have relatively fast activation time (tens of milliseconds). These are virtually insensitive to cAMP. HCN2 channels are expressed most extensively and found in most parts of the brain but are predominant in the sub-cortical areas (e.g. thalamus and brainstem). It has intermediate (several hundreds of milliseconds) activation time and a depolarizing shift in its voltage dependence on exposure to cAMP. HCN3 has diffuse but low level distribution in the brain except for the olfactory bulb and some hypothalamic nuclei where HCN3 expression level is high. HCN4 is a subtype present mostly in thalamic relay neurons, basal ganglia and olfactory bulb. Both HCN3 and HCN4 have slow activation time. Like HCN2 channels, HCN4 are also strongly modulated by cAMP whereas, HCN3 are only weakly modulated by cAMP [62,63,70,71].

As like that of other ion channels, HCN are widely expressed in central as well as peripheral nervous system. These channels perform various functions such as control of membrane resting potential, dendritic integration and neuronal pacemaking. Therefore, even subtle change in their subunit composition, their expression level or localization to sub-cellular compartment may lead to disease condition. Channelopathies for two major HCN channels (HCN1 and HCN2) have not been reported so far in human patients suffering from epilepsies. Therefore, the current knowledge on the pathophysiological importance of HCN channels are by and large is based on studies on animals and has yet to be confirmed in human beings [71].

Dysregulation of HCN channel expression and aberrant HCN channel function have been associated with various types of idiopathic and acquired epilepsies. Deletion of HCN1 subtype in mice exerts a proconvulsive effect and accelerates epileptogenesis [63,72]. Loss of HCN1 channel expression and function also occurs during epileptogenesis in animal models of acquired epilepsy, contributing to neuronal hyperexcitability and promoting further seizures [73]. Genetic deletion of HCN2 channel subtype has the typical clinical hallmarks of absence epilepsy [74]. Therefore, the results from genetic mouse models suggest that suppression of Ih is involved in the generation of neuronal hyperexcitability. However, there is growing evidence that not only down-regulation but also enhancement of HCN channel mediated current may contribute to pathology of seizure. For example, an HCN2 variant (where residue 719-721 are deleted) was indentified in patients suffering from febrile seizure; when heterologously expressed, this variant leads to 35% larger current than control [71,75]. Following the rat model for febrile seizure, the CA1 pyramidal neurons exhibit enhanced dendritic Ih. This is accompanied by altered gating properties; down-regulation of HCN1 and upregulation of HCN2. It cannot be considered as primary cause for epileptic event but the data indicated that the transcriptional changes in HCN channels were pathologically associated with this form of epilepsy [76]. The WAG/Rjj rat model of absence epilepsy showed a loss of HCN1 function in the cortex [77] but enhanced HCN1 expression in the thalamus [78]. Finally, in addition to the absolute expression levels, the subunit composition of heteromeric channels (e.g. HCN1: HCN ratio), their stoichiometry and their targeting neuronal compartment can play a crucial role for inducing aberrant neuronal firing [71].

The complexity and diversity in connecting the role of impaired HCN channel to epilepsy makes it very difficult and challenging to develop a generally applicable rationale for designing newer anticonvulsant drugs acting through HCN channels. There are reports suggesting either pharmacological block or activation of HCN channel may be beneficial in controlling epilepsy depending on the clinical circumstances. Currently

available antiepileptic drugs like Lamotrigine (voltage gated sodium channel blocker) [63], Gabapentin (Calcium channel blocker) [79] and Acetazolamide (Inhibitor of carbonic anhydrase)[80] were shown to upregulate the activity of HCN channels. The contribution of HCN channel upregulation to the clinical effect of these drugs in currently unclear. However, it may be considered that the action of these drugs is directed primarily at HCN1. ZD7288 (HCN channel inhibitor) which was hypothesized to possess pro-convulsant effect conversely, showed reduction in the generation of hippocampal epileptic discharges in rabbit [81]. Therefore, use of HCN channel blockers or activators depends on the type of epilepsy and expression levels of different subunits of HCN channels. Three crucial points should be considered while developing newer drugs acting on HCN channels. First and most important goal will be to design subtype specific blocker or activator e.g. compounds inhibiting HCN1 could be used for the treatment of epilepsy but would not have major effect on cardiac pacemaker functions[82]. Secondly, as most HCN channel modulators target intracellular side (ivabradine) of HCN channels they would require drug penetration through plasma membrane which may get trapped inside ion conducting pore (ZD7288). This trapping may either lead to very slow elimination of drug from conducting pore or may cause irreversible blocking of these channels. Therefore, it is desirable to design compounds that bind to HCN channels in a reversible manner and at the extracellular side of the membrane [72]. Development of compounds with higher affinity is also one of the important considerations in the AED development. The currently available channel agonist or antagonists have unpretentious IC50 values in the low micro-molar range. Ideally it should operate in the nano-molar range so that risk of off-target and toxic side effect of drugs can be avoided [72].

Conclusion

Much of the research work done on various genetic and acquired models of epilepsy as well as human tissue of epileptic patients revealed that ion channelopathy is proved to be one of the causes of human epilepsy. Various ion channel modulators have been demonstrated to be potent AEDs. Despite this fact, about 30% patients are resistant to currently available AEDs and continue to suffer from epilepsy. Thus, there is a dire need for developing newer AEDs to overcome these therapeutic difficulties.

Various animal experiments as well as genetic studies on epileptic patients strongly indicate that some forms of epilepsy may have sodium channel alteration in its pathogenesis. Design and development of sodium channel blockers with better efficacy and lesser side effects can be achieved by sodium channel subtype specificity in particular. At the same time voltage and use dependent blocking of the sodium channels along with multiple mechanisms on other important channels can also be studied for better therapeutic outcome. Voltage gated potassium channels are responsible for repolarisation of cell after depolarization. Therefore, pharmacological activation of Kv could reduce neuronal excitability and serve as good target for development of new AEDs. A number of potassium channel mutation studies and other animal models have shown that Kv like 1, 3, 4 and 7.2/7.3 in one or the other way are associated with epilepsy which could be targeted.

Calcium channels play significant role in cell membrane depolarization, controlling intracellular signalling and biochemical pathways. As these channels are very complex and wide spread, a small alteration in their biophysical property or their expression may induce epileptic seizures. Development of LVA calcium channels blockers or HVA calcium channel activators could prevent hyper-bursting in thalamo-cortical network and the absence seizures.

HCN channels are non-selective cation channels present in cardiac tissue and neurons. They play significant but complex role in fine tuning of cellular and network activity. Therefore, dysregulation of HCN channel could be responsible for seizure as shown by various animal

models. Subtype specific activators and blockers of HCN channels could be designed for treatment of epilepsy depending on type of epilepsy and expression of various subtypes of HCN channels in different regions of brain.

The new generation of ion channel modulators would have a great potential in design and development of efficacious drugs in the treatment of epilepsy.

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