Channelopathy of Dravet Syndrome and Potential Neuroprotective Effects of Cannabidiol

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Journal of Central Nervous System Disease
Volume 13: 1–14

DOI: 10.1177/11795735211048045
SAGE

ABSTRACT

Dravet syndrome (DS) is a channelopathy, neurodevelopmental, epileptic encephalopathy characterized by seizures, developmental delay, and cognitive impairment that includes susceptibility to thermally induced seizures, spontaneous seizures, ataxia, circadian rhythm and sleep disorders, autistic-like behaviors, and premature death. More than 80% of DS cases are linked to mutations in genes which encode voltage-gated sodium channel subunits, SCN1A and SCN1B, which encode the Nav1.1α subunit and Nav1.1β1 subunit, respectively. There are other gene mutations encoding potassium, calcium, and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels related to DS. One-third of patients have pharmacoresistance epilepsy. DS is unresponsive to standard therapy. Cannabidiol (CBD), a non-psychoactive phytocannabinoid present in Cannabis, has been introduced for treating DS because of its anticonvulsant properties in animal models and humans, especially in pharmacoresistant patients. However, the etiological channelopathiological mechanism of DS and action mechanism of CBD on the channels are unclear. In this review, we summarize evidence of the direct and indirect action mechanism of sodium, potassium, calcium, and HCN channels in DS, especially sodium subunits. Some channels' loss-of-function or gain-of-function in inhibitory or excitatory neurons determine the balance of excitatory and inhibitory are associated with DS. A great variety of mechanisms of CBD anticonvulsant effects are focused on modulating these channels, especially sodium, calcium, and potassium channels, which will shed light on ionic channelopathy of DS and the precise molecular treatment of DS in the future.

KEYWORDS: Dravet syndrome, cannabidiol, sodium channel, potassium channel, calcium channel, HCN

TYPE: Review

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

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Introduction

Epilepsy is the fourth most prevalent neurological disorder after strokes, Alzheimer's disease and migraine, and occurs in approximately 65 million people worldwide.[1] About 70% of the patients achieve seizure freedom with antiepileptic drugs (AEDs), while 30% have drug-resistant epilepsy.[2] Epilepsy is defined as a disorder with either recurrent, unprovoked seizures (at least 2 or more unprovoked seizures occurring at least 24 hrs apart) or an increased tendency toward recurrent unprovoked seizures in the next 10 years (1 unprovoked seizure along with additional clinical, radiological, or electroencephalographic (EEG) evidence suggests at least 60% risk for future seizures) or when an epilepsy syndrome is diagnosed.[3] Dravet syndrome (DS), also known as severe myoclonic epilepsy of infancy, was described in 1978 by Charlotte Dravet, it occurs more often in males than in females (2:1).[4,5] DS is a channelopathy, neurodevelopmental epileptic encephalopathy which is not a pure consequence of epilepsy but rather arises directly from the effect of the genetic mutation, eventually modulated by other genetic and no genetic factors.[6] While the term channelopathy implies defects in the pore-forming subunit of the voltage-gated sodium, calcium, or potassium signaling complex, the non-pore–forming components are also critical in physiology and disease. Neuronal channelopathies cause various brain disorders including epilepsy, migraine, and ataxia. At least 80% cases of DS are linked to mutations in genes which encode voltage-gated sodium channel (VGSC) subunits, Sodium Voltage-Gated Channel Alpha Subunit 1 (*SCN1A)* and *SCN1B*, which encode the Nav1.1α subunit and VGSC β1 subunit, respectively.[7] There are also other genes mutations encoding potassium and calcium channels related to DS. Some genes mutations show loss-of-function (LoF) or gain-of-function (GoF) mutations resulting in channel inhibition or hyperactivity in interneurons or excitatory neurons, which lead to imbalance of excitation vs inhibition in neural circuits contributing to the etiology of DS.

As mentioned, DS is an early-onset epileptic encephalopathy characterized by seizures, developmental delay, and cognitive impairment and is also susceptible to thermally induced seizures and spontaneous seizures, ataxia, circadian rhythm and sleep disorders, autistic-like behaviors, and premature death.[8–11] At

present, the treatment strategy for this order may benefit from epilepsy surgery; however, this procedure is only used in a subset of cases.[12] Though there are a number of anticonvulsant drugs available, there are no antiepileptogenic drugs that mitigate the progression of the disease. DS is also unresponsive to standard therapy. The current first-line therapy for DS is a combination of clobazam and valproic acid. Stiripentol (STP) is often added for pharmacoresistant patients but is not an FDA-approved treatment. Unfortunately, this combination not only fails to provide complete seizure control, but also causes serious adverse events in over 50% of patients.[13,14] Although a big progress on the genetic diagnosis study on DS has been made, effective therapy for DS is extremely limited. Therefore, there is an urgent need to develop alternative treatments.

Since 1970, marijuana has been listed as a Schedule I drug in the United States under the Controlled Substances Act, a classification that indicated it as a substance with high abuse potential and with no currently accepted medical use. Cannabidiol (CBD) was first isolated from marijuana extract in 1940, but no further major study was reported on it for the next 25 years.[15] In an *SCN1A* knockout mouse model of DS, CBD alone treatment can decrease not only the number of spontaneous seizures but also the duration and severity of thermally induced seizures, and autistic-like social interaction deficits improved with low dose CBD but, interestingly, not with the higher dose of CBD required for seizure control.[16] Cognitive impairment is a frequent comorbidity affecting 75% of people with epilepsy including patients with DS which results from LoF mutations in VGSC gene *SCN1A*. Therapy for cognitive impairment would dramatically improve the lives of these patients, substantially reduce long-term care costs, and reduce accidental deaths. Chronic administration of CBD prevents premature mortality and improves several behavioral comorbidities, including impaired cognition and social interaction, associated with the *SCN1A*+/− mouse DS model.[17,18] Interestingly, plant-derived, highly purified CBD in a sesame oil-based oral solution has antiseizure properties in a broad range of epilepsy syndromes and encephalopathy, including DS, Lennox–Gastaut syndrome, and tuberous sclerosis complex.[19] Recent pre-clinical and clinical evidence suggests that CBD may provide an effective, tolerable alternative to current therapeutics on DS, and CBD has been approved in the treatment of DS and Lennox–Gastaut syndrome in USA, Europe, and Australia in 2018,2019, and 2020, respectively.[20–22] However, the action mechanism of CBD is unclear, Therefore, we will summarize the effect of CBD on sodium, calcium, and potassium ion channels in DS and other possible therapeutic mechanisms on DS.

Sodium Channels

Voltage-gated sodium channels (Navs) were discovered by Hodgkin and Huxley in 1952.[23] Mammalian VGSC are composed of a large pore-forming unit that associates with 1 or 2 subunits and have been found in almost every type of neuron examined. Voltage-gated Na^+ channels in the brain are complexes of an α subunit containing the voltage sensor and ion-conducting pore, in association with auxiliary β subunits (β1–β4), which modify the kinetics and voltage dependence of gating and serve as cell adhesion molecules. Four functional VGSC α subunits are expressed in adult mammalian brain: Nav1.1, Nav1.2, Nav1.3, and Nav1.6 channel subtypes, encoded by the *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A* genes, respectively, each domain composed by 6 transmembrane segments (S1-S6), and 1 or more β subunits associated by noncovalent interactions or disulfide bond.[24] Several mutations in Nav channel genes have been associated with epilepsy.[25] Sodium channel α subunits are encoded by 10 genes, which are expressed in different excitable tissues. Nav1.1, Nav1.2, Nav1.3, and Nav1.6 are the primary sodium channels in the central nervous system (CNS). Nav1.7(*SCN9A*), Nav1.8(*SCN10A*), and Nav1.9(*SCN11A*) are the primary sodium channels in the peripheral nervous system. Nav1.4 is the primary sodium channel in skeletal muscle, which causes contractility complications, including myotonia and periodic paralyzes, whereas Nav1.5 (*SCN5A*) is primary in the heart.[26] As for 4 Navβ subunits in total, β1 and β3 are associated noncovalently with α subunits and resemble each other most closely in amino acid sequence, whereas β2 and β4 form disulfide bonds with α subunits and also resemble each other closely.[27] Mutations in *SCN1A*, *SCN1B*, *SCN5A*, and *SCN8A* genes are associated with increased risk of early mortality and estimates of sudden unexpected death in epilepsy (SUDEP) incidence in DS reach 10% or greater. Intriguingly, mutation of *SCN1A* has now been associated with sudden infant death syndrome (SIDS) in a small cohort.[28] We will categorize sodium channel subunits based on their genes' encoding.

SCN1A Gene

The *SCN1A* gene encodes for the α subunit of Nav1.1 and is allocated at the 2q24.3 chromosome between 165,984,641 and 166,149,161 base pairs. The Nav1.1 channel protein encoded by the *SCN1A* gene is a ~260kD protein divided into 4 near-homologous homomeric domains (I-IV). Within each domain are 6 transmembrane domains (S1-S6) including an S4 voltage sensor, an S3-S4 intracellular loop that folds to become the inactivation gate, and an S5-S6 extracellular linker domain that translates to a hairpin-like loop integrated into the channel pore.[29] Nav1.1 is widely expressed in the cortex and hippocampus of the CNS, predominant in inhibitory γ-aminobutyric acid (GABAergic) interneurons, particularly in parvalbumin-positive fast-spiking basket cell interneurons (PV-INs), regulating neuronal excitability, decreased synaptic inhibition, hyperexcitability, and epileptic diseases due to imbalance between excitation and inhibition. Nav1.1 mutation leads to preferential dysfunction of interneurons, and epilepsy in DS model.[30] All these support the theory of "interneuron hypothesis."[31] Among

the Nav subtypes related to epilepsy, Nav1.1 is doubtless the most relevant with more than 1500 mutations, and *SCN1A* mutations account for almost 80% of DS.[32] Truncation and missense mutations are the most observed alterations. Local ablation of Nav1.1 channel in the hippocampus and cortex in mice results in focal seizure activity that can generalize, which indicates that spontaneous epileptic activity may initiate in multiple brain regions, and hippocampal deletion of Nav1.1 channel in mice mimicked the thermal seizures and cognitive deficit characteristic of DS.[33,34] Mouse genetic models that implicate specific loss of sodium currents and action potential firing in GABAergic inhibitory interneurons as the fundamental cause of DS.[35] *SCN1A*+/- heterozygous mutation targeting, *SCN1A* haploinsufficiency to Nav1.1 channel, induces LoF of Nav1.1 channel, which reduces sodium current and excitatory drive in many types of GABAergic neurons such as parvalbumin, somatostatin (SST), and neuropeptide-Y (NPY)-type in forebrain GABAergic neurons. Hypoexcitability of these interneurons is sufficient in causing the DS well-defined epileptic phenotype.[9,36,37] Electrophysiological recordings in acute brain slices prepared from *SCN1A*+/- mice indicate that parvalbumin interneurons (PV-INs) rely on Nav1.1 for action potential generation and hence are dysfunctional in DS.[38] A third major class of interneurons expressing vasoactive intestinal peptide express Nav1.1 and are dysfunctional in DS.[39] In contrast, targeting *SCN1A* mutation to excitatory neurons ameliorate DS in mice, which is opposite to the effects of gene disruption in inhibitory neurons.[40] Of interest, phenotype severity in *SCN1A*+/- mice is strongly dependent on strain background. *SCN1A*+/- mice on the resistant 129 strain background (129.*SCN1A*+/-) have no overt phenotype and live a normal lifespan. In contrast, *SCN1A*+/- mice on a (129xB6) F1 strain background (F1. *SCN1A*+/-) exhibit spontaneous seizures, severe epilepsy, and premature lethality, with 50% dying by 1 month of age, and age-dependent hippocampal neuron sodium currents also correlate with epilepsy severity such as premature lethality in DS.[41,42] The Collaborative Cross (CC) is a large panel of recently established multi parental recombinant inbred mouse lines, specifically designed to overcome the limitations of existing mouse genetic resources for analysis of phenotypes caused by combinatorial allele effects. Collaborative Cross mice crossed with *SCN1A*+/- mice to further explore the strain-dependent difference in phenotypes. Phenotype frequently does not correlate with genotype, although mutations in the pore-forming region of Nav1.1 often predict poorer clinical outcomes.[43] Loss of Nav1.1 channel with conditional deletion of *SCN1A* in forebrain GABAergic neurons is both necessary and sufficient to cause epilepsy and premature death in DS.[44] There are 3 heterologous expression systems' models used to investigate the cellular consequences of *SCN1A* mutations linked to epilepsy including mouse, zebrafish, and fruit fly models.[45] *Drosophila* knock-in flies with the K1270T *SCN1A* missense mutation model of DS reveals a constitutive and conditional reduction in sodium current.[46] Conversely, not all *SCN1A* mutations mimic epileptic encephalopathies such as DS.[11,32] Sodium channel blockers (e.g.,, carbamazepine, oxcarbazepine, lamotrigine, and phenytoin) and the γ-aminobutyric acid (GABA) transaminase inhibitor vigabatrin are not effective or even exacerbated seizures in humans and mice with *SCN1A*+/- mutations.[47] Overall, clobazam is the most effective anticonvulsant in *SCN1A*+/- mice, consistent with its effect in DS.[48] However, GS967, a potent, unconventional sodium channel blocker, significantly improved survival of *SCN1A*+/- mice, and suppressed spontaneous seizures by involving a secondary change in Nav1.6.[49] Pathogenic variation in *SCN1A*, the prototypical sodium channel gene, also underlies a febrile seizure phenotype ranging from mild genetic epilepsy with febrile seizures plus syndrome (GEFS+) to treatment resistant DS. Human and experimental research show that pathogenic variation in *SCN1A* is a risk factor for SUDEP in about 4% of individuals affected by DS. *SCN1A* GoF with Nav1.1-p.T226M patients may benefit from AED that reduce sodium current, which are relatively contraindicated for patients with traditional DS but serve as standard of care for patients with GoF *SCN1A* Early Infantile Encephalopathy (EIEE).[50] An analysis of genotype-phenotype correlations in children with DS show that the correlation is predictably more complex for interpreting missense variants, as not only the location but also the nature of the amino acid substitution impact disease phenotype than truncation, and missense variants in *SCN1A* were most common in the sodium voltage-sensor and pore domains.[51]

SCN1B Gene

SCN1B gene encodes VGSC β1 subunit.[7] *SCN1B* is expressed in both the brain and heart. β1 regulates gating and kinetics of the ion channel pore, functions as a cell adhesion molecule (CAM), and initiates cell signaling. While the majority of DS cases are linked to *SCN1A* haploinsufficiency, *SCN1B* homozygous mutations coding for Navβ1 are also linked to DS.[52] *SCN1B*-/- mice have a DS phenotype with SUDEP. *SCN1B* are developmentally regulated cell adhesion molecules and ion channel modulators that play critical roles in the regulation of excitability. Mutations in the genes encoding β subunits of VGSCs are linked to a number of diseases, including epilepsy, sudden death syndromes like SUDEP and SIDS, and cardiac arrhythmia.[53] Mutations in *SCN1B* are associated with the genetic epilepsy with febrile seizures plus (GEFS+) spectrum disorders in humans, and *SCN1B*-null mice display severe spontaneous seizures and ataxia from postnatal day 10 (P10).[54] Pathogenic LoF variants in *SCN1B* are linked to DS. *SCN1B* p.R125C is an autosomal recessive cause of DS through functional gene inactivation.[55] *SCN1A*+/- mice treated with adeno-associated virus-Navβ1 showed reduced spontaneous seizures and normalization of motor activity through direct sodium channel potentiation and/or modulation of potassium channels (KV4.2).[56] *SCN1B*-/- mice have cell type specific

changes in Na^+ (INa) and K^+ (IK)currents. In addition, *SCN1B-/-* mice have neuronal proliferation, migration, and pathfinding defects at postnatal day 5 (P5) that precede seizure onset at ~P10. Defective cell adhesion in *SCN1B*-linked DS may not contribute to seizures but instead impact other comorbidities. *SCN1B-/-* mice also have delayed maturation of neuronal Cl^- gradients such that GABAergic signaling remains depolarizing and excitatory until ~P17-18, which may contribute to hyperexcitability in *SCN1B*-linked DS.[54,55]

SCN2A Gene

Nav1.2 is encoded by the *SCN2A* gene. It is located on chromosome 2q24.3 and expressed in the CNS, especially in excitatory neurons and glutamatergic neurons.[57] GoF mutations of *SCN2A* are related to epilepsy because it causes neuronal hyperexcitability; and many of these refractory epilepsies are believed to be manifestations of mutations in *SCN2A*, the gene for the human VGSC hNav1.2.[58] GoF variants (such as M1879T or R1882Q) in *SCN2A* are thought to cause early-onset epilepsy (onset before 3 months of age) by promoting excitability of cortical neurons during the developmental stage when Nav1.2 predominates in the axon initial segment (AIS).[59] However, LoF SCN2A gene mutations for epilepsy are related to late-onset epilepsy.[60] Interactions between genetic variants of *SCN2A* and *KCNQ2* in the mouse and variants of *SCN1A* and *SCN9A* in patients provide models of potential genetic modifier effects in the more common human polygenic epilepsies.[61] Variants in *SCN2A*, *KCNQ2*, and *SCN8A* can dramatically influence the phenotype of mice carrying the *SCN1A-R1648H* mutation and suggest that ion channel variants may contribute to the clinical variation seen in patients with monogenic epilepsy.[62] Epilepsy mutations in this protein are generally believed to cause a net augmentation of the channel function, leading to hyperexcitability and inappropriate action potential firing. Human epilepsy patients with channelopathies such as DS and GEFS+ reveal that Nav1.1 is the dominant channel in inhibitory circuits while Nav1.6 and Nav1.2 are the dominant channels in excitatory pyramidal neurons.

SCN3A Gene

Type 3 voltage-gated Na^+ channel, a subunit, the Nav1.3, is encoded by *SCN3A*. The *SCN3A* gene is located on human chromosome 2q24, in a cluster with *SCN1A* and *SCN2A*.[63] Nav1.3 is expressed predominantly in the CNS during embryonic and neonatal development, being extremely low or sometimes undetectable in postnatal individuals. Heterozygous variants of *SCN3A* in association with moderate forms of epilepsy, and homozygosis is related with severe cognitive damage and premature mortality, resulting in a broad range of epileptic phenotypes. Both GoF and LoF may lead to an increased seizure susceptibility.[64] Mutation of sodium channel *SCN3A* potentially relates to cryptogenic pediatric partial epilepsy.[63] LoF of *SCN3A* caused by reduced protein expression or deficient trafficking to the plasma membrane may contribute to increased seizure susceptibility.[65] Novel missense *SCN3A* variants (R357Q, D766N, E1111K and M1323V) associated with focal epilepsy in children.[66] As an important regulator of neuronal excitability in the developing brain, *SCN3A*, encoding Nav1.3, is known to be highly expressed in the brain, and linked to early infantile epileptic encephalopathy.[67] An immunocytochemical survey also revealed as specific upregulation of Nav1.3 channels in a subset of hippocampal interneurons, but this upregulation was insufficient to compensate for the loss of the sodium current of Nav1.1 channel.[68]

SCN8A Gene

The *SCN8A* gene encodes for type 8 voltage-gated Na^+ channel a subunit, the Nav1.6, located in chromosome 12q13.13, which is involved in action potential generation. Nav1.6 is the primary sodium channel in excitatory neurons, where it drives repetitive firing. The first case of *SCN8A* pathogenic variant associated with epilepsy was reported 8 years ago.[69] *SCN8A* mutation contributes to 2 distinct seizure phenotypes: (1) hypoexcitation of cortical circuits leading to convulsive seizure resistance, and (2) hyperexcitation of thalamocortical circuits leading to non-convulsive absence epilepsy.[70] *SCN8A* is also related to epilepsy and approximately 100 mutations have been reported in patients with severe Early Infantile Epileptic Encephalopathy subtype 13 (EIEE13). This disease mechanism is reflected by the therapeutic response of VGSC blockers. Various clinical reports have shown that *SCN8A*-related epilepsy patients benefit from VGSC blockers, contrasting their inefficacy, or even detrimental effects in DS.[71] Reports have suggested that patients with *SCN8A*-related epilepsy have increased risk of SUDEP, ranging from 1% to 10%.[72] Reduction of *SCN8A* transcript through an antisense oligonucleotide by 25–50% delayed seizure onset and lethality in mouse models of *SCN8A* encephalopathy and DS.[73] A small number of mutations have been found in *SCN2A, SCN3A,* and *SCN9A*, and studies in the mouse suggest that *SCN8A* may also contribute to seizure disorders. An SCN8A mutation with heterozygous and homozygous *SCN8A-R1627H* mutants can both confer seizure protection and increase seizure susceptibility.[74] Nav1.6 is 1 of the 2 main sodium channels expressed in pyramidal neurons, which are responsible for excitatory signals via glutamate excretion. Selective inhibition of Nav1.6 could be just as efficient as selective activation of Nav1.1 in two CRISPR/Cas9-generated knockout zebrafish models or *SCN1A*-related epilepsies and these approaches could prove to be novel potential treatment strategies for DS and other genetic epilepsies.[75] The GoF mutations in Nav1.6 cause channel hyperactivity due to augmented excitability and firing rates of pyramidal cells concurrent with an increase in glutamate release. Therefore, GoF mutations in *SCN8A* can lead to a severe epileptic encephalopathy subtype by over activating Nav1.6 channels. Heterozygous LoF mutations of *SCN8A*

cause intellectual disability with or without seizures.[76] *SCN8A* should be considered as a candidate gene for intellectual disability, regardless of seizure status variants in *SCN8A*, accounting for 1% of known epileptic encephalopathies.[77] A recent study of a DS model using zebrafish demonstrated the use of the channel blocking compound, MV1312, which is 5–6 fold selectivity of Nav1.6 over Nav1.1–1.7, reduced burst movement phenotype and the number of epileptiform events, activity similar to that described with the use of a selective Nav1.1 activator AA43279.[75] GS967, a potent and unconventional sodium channel blocker, is a Nav1.6 modulator that inhibits the persistent sodium current and exhibits a protective effect, which shows that a significantly improved survival of *SCN1A*+/- mice and suppressed spontaneous seizures by involving a secondary change in Nav1.6.[49,78]

SCN9A Gene

The *SCN9A* gene encodes for the Nav1.7 channel, located in chromosome 2q24.[61] Nav1.7 is expressed preferably in the peripheral nervous system (PNS), but it is also expressed in the CNS.[79] Some Nav1.7 mutations, as a modifier gene, could probably contribute to complex inheritance for these unexplained cases of DS.[80] A follow-up study of 102 patients with DS identified 7 patients with mutations in both *SCN1A* and *SCN9A*. The *SCN9A* variants may modify the severity of DS in these patients with primary mutations in *SCN1A*.[81]

The Mechanism of CBD on Sodium Channels

Although CBD was approved under the brand name Epidiolex in June 2018 by the FDA for the management of DS and Lennox–Gastaut syndrome (LGS).[82] In September 2018, the US Drug Enforcement Administration determined that CBD would be a Schedule V medication,[83] but its action mechanism is not clear. Some clinical trials found that there was a greater prevalence of seizure worsening when CBD was used in patients with LGS syndrome who were not taking clobazam and in patients with DS who were not taking clobazam and STP. Nonselective sodium channel blockers are well recognized to aggravate seizures in DS and are contraindicated in the condition.[84,85] Several pharmacokinetic drug-drug interactions such as CBD and clobazam shown that CBD neuroprotective effect due to increase in plasma concentrations of norclobazam, an active metabolite of clobazam by its inhibition of CYP2C19(Cytochrome P450 2C19, an enzyme protein).[86,87] CBD and clobazam together enhanced inhibitory $GABA_A$ receptor activation.[88] Interestingly, CBD is more potent at inhibiting CYP3A4-mediated metabolism of clobazam than CYP2C19-mediated metabolism of norclobazam.[49] Furthermore, the cumulative data suggest that CBD has the independent antiseizure effect irrespective of concomitant clobazam.[89,90] CBD as adjunctive treatment in patients with DS has also been associated with improvement in global functioning measures and no significant changes in sleep disruption, daytime sleepiness, quality of life, and behavioral adaption.[91] As for the efficacy and safety in the treatment of patients with DS, clinical data suggest that adverse events are significantly associated with adjunctive CBD were somnolence, decreased appetite, diarrhea, and increased serum aminotransferases.[92] CBD is also highly lipophilic and readily crosses the blood-brain barrier. At steady state, the time to peak plasma concentration occurs between 2.5 and 5 hours, and administration with a high-fat, high-calorie meal increases the maximal plasma concentration. CBD has a large volume of distribution, ranging from 20 963 to 42 849 L and is > 94% protein bound.[93] Metabolism occurs predominantly via the liver through CYP2C19, CYP3A4, UDP glucuronosyltransferase 1A7 (UGT1A7), UGT1A9, and UGT2B7. There is 1 active metabolite, 7-OH-CBD, which is metabolized to the inactive metabolite, 7-COOH-CBD. CBD is almost exclusively excreted in the feces. The half-life is 56 to 61 hours.[93] In vitro, studies have shown that CBD has a direct sodium channel modulation[94] and blocks voltage-gated sodium channel[105]; CBD has also been found in patch clamp recordings to be a non-selective inhibitor of recombinant VGSCs at concentrations that could be relevant therapeutically.[95] In detail, these studies were performed using different in vitro models as rat brain slices, cultured mouse cortical neurons, and human SH-SY5Y cell culture. CBD was tested (1–10 μM) on 2 different types of VGSCs: the Nav1.1 and Nav1.2 subtype, respectively.[95] While a single report described the modulation of resurgent Nav current by CBD[96] and another described the inhibition of Nav channel function at concentrations higher than clinically relevant.[95] Conversely, the lack of effect of purified CBD on peak transient current and lack of use-dependent block has been reported.[97] CBD appeared to inhibit and block the opening of Nav1.1 to Nav1.7 with low μM potencies, measured in human cell culture and rat brain slices.[98,99] CBD can preferentially target abnormal/mutant sodium channels, which would be of interest in, for example, DS.[100] The action of CBD on voltage-gated sodium channels, mainly on Nav1.1, 1.2, and 1.6, were summarized in the following:

1. CBD (albeit at high doses) protects against thermally induced seizures (modeling febrile seizures) in a SCN1A+/– mouse model of DS.[16] Lowering GABAergic activity related to the NaV1.1 LoF with M145T SCN1A LoF mutation is also related to Mesial temporal lobe epilepsy (MTLE).[101] CBD was capable of increasing GABA current amplitude in NaV1.1 of MTLE patients.

2. CBD (1 μM) preferentially inhibits resurgent currents over transient currents in human embryonic kidney (HEK) cells stably expressing wild type hNav1.2 channels.[102] Due to the role of Nav1.2 and Nav1.6 in excitatory neurons, preferentially inhibition in resurgent

currents by CBD could be speculated to possibly reduce the excitability in that subset of neurons and decrease the frequency of seizures by a change in threshold of activation and repetitive fire.[103]

3. CBD was able to preferentially target and inhibit aberrant and the increased resurgent currents in mutations in Nav1.6. A study demonstrated that CBD at 1 μM inhibit preferably resurgent currents than transient current in Nav1.6 wild type (WT) and also inhibit peak resurgent current in Nav1.6 mutant N1768D, with less effect in current density and without altering voltage dependence of activation.[105] Possibly the modulation of CBD over mutations in SCN8A that promotes a phenotype with increased resurgent currents would cause a reduction in the causative excitability of epileptic seizures. The enhancement of resurgent current by the SCN8A/Nav1.6 epilepsy mutation N1768D can also be selectively inhibited by CBD.[104,105] CBD also inhibits resurgent current more than transient current associated with 2 epilepsy associated SCN8A variants (L1331V, N1768D).[105]

Intriguingly, θ-γ coupling may serve as an early indicator of inhibitory dysfunction and seizure risk in DS and θ-γ coupling reduction in DS mice model was partly restored by CBD.[106] Another interesting example is the finding that overexpression of beta-amyloid peptide, the pathogenic amyloid-forming fragment in Alzheimer disease (AD), drives down SCN1A expression in cortical interneurons.[107] This secondary SCN1A lesion may contribute in part to the hyperexcitability identified in both mouse models and patients with AD dementia.[108]

Potassium Channels

There are more than 80 potassium channels, of which 10% are associated with epilepsies in human and animal seizure models. Structurally, Potassium (K) channels consist of transmembrane (TM) protein elements similar to voltage-gated calcium channel family (Cav) and sodium (Nav) channels. Four α subunits are necessary to build a functional K channel, and form heteromers with β subunits. K channels are categorized as inward rectifier potassium channels (Kir), 2 pore dominant potassium channels (K2p), voltage-gated potassium channels (Kv), and calcium-dependent potassium channels (Kca). Kca are further classified as small, intermediate, and big-conductance, that is, SK1-3 (Kca2.1, Kca2.2, and Kca2.3), IK (Kca3.1), and BK (Kca1.1) channels, respectively.[109] K^+ channels at the AIS dampen near-threshold excitability of neocortical fast-spiking GABAergic interneurons.[110] Both 4-Aminopyridine (4-AP; via blockade of Kv3 channels in PV-INs) and picrotoxin (as a non-competitive blocker of $GABA_A$ receptors) impair inhibition, which contributes to their antiseizure-like activity.[111]

Potassium Voltage-Gated Channel Subfamily A Member 2 (*KCNA2*) joins a growing list of voltage-gated potassium channel genes associated with epileptic encephalopathy, including Potassium Voltage-Gated Channel Subfamily Q Member 2 (*KCNQ2*), *KCNQ3*, Potassium Sodium-Activated Channel Subfamily T Member 1 (*KCNT1*) and Potassium Voltage-Gated Channel Subfamily B Member 1 (*KCNB1*). *KCNA2* encodes KV1.2, a voltage-gated potassium channel subunit that contributes to repolarization of the neuronal membrane following an action potential. Ion channel mutations have been implicated as a major cause of developmental and epileptic encephalopathies such as Epilepsy of Infancy with Migrating Focal Seizures (EIMFS). EIMFS is a rare, developmental, and epileptic encephalopathy (DEE) presenting within 6 months of life with polymorphous, migrating focal seizures. In particular, EIMFS is commonly associated with GoF mutations in *KCNT1* (slack, KNa1.1), a gene that encodes a neuronal sodium-gated potassium channel subunit. Numerous mutations and 1 deletion within *KCNT1* have been described as causative of EIMFS.[112] Complete absence of KV1.2 in homozygous mice resulted in spontaneous seizures and premature death, and heterozygous deletion resulted in increased seizure susceptibility.[113] $KCNB1^{G379R}$ mice recapitulate many features observed in individuals with developmental and epileptic encephalopathies (DEE) due to pathogenic variants in *KCNB1* which encodes KV2.1.[114] Mutations of *KCNB1*-(G379R, S347R, T374I) KV2.1 channel result in the early onset epileptic encephalopathy.[115] Voltage-gated potassium channel (KV2.1) functional defects caused by *KCNB1* variants are associated with DEE.[116] The *KCNB1*-I199F variant exhibited a function relative to the wild-type channel which suggests the possibility that the degree of *KCNB1* protein dysfunction may influence disease severity.[117] Potassium Voltage-Gated Channel Modifier Subfamily V Member 2 (*KCNV2*, Kv8.2) contributes to epilepsy susceptibility. An early onset epileptic encephalopathy syndrome can be caused by a gain of function mutation in the potassium channel *KCNT1* and *KCNT1* partial antagonist quinidine is effective to mitigate the seizure.[118] The null mutation of *KCNA1*, which encodes voltage-gated Kv1.1 potassium channel α-subunits in the mouse, results in juvenile lethality that appears to result from seizures.[119] The *KCNQ2* channel is part of a complex that produces a slowly inactivating potassium current that limits neuronal firing rates. Impaired *KCNQ2* activity would be expected to increase neuronal firing. Moreover, the double heterozygotes' mice for a mild mutation of *SCN2A* and a subclinical mutation of *KCNQ2* were generated, the genetic interaction between *SCN2A* and *KCNQ2* resulted in severe seizures and the mice died from status epilepticus (SE) within 3 weeks after birth. This dramatic example of gene interaction may help to elucidate the functions of these 2 channels.[120]

Loss of calcium-activated potassium SK channels' activity causes the reticular thalamic neurons to become hyperexcitable and promote non-convulsive seizures in DS.[121] BK channels are Ca^{2+} and voltage-activated potassium channels with large conductance. In the brain, BK channels are expressed in a large

variety of neurons and have diverse functions, which include controlling the action potential shape, regulating firing frequency, and regulating neurotransmitter release. Once BK channels are calcium and voltage-activated receptors, as well as potassium selective, increasing BK channels activity, may lead to increase in potassium conductance, which in turn reduces membrane potential, while its blockade can reduce the spike broadening during a burst.[122,123] These channels have been shown to mediate rapid spike repolarization and fast after-hyperpolarization (fAHP) in many types of neurons. It was discovered that an abnormal increase in the BK channel conductance, caused by a GoF mutation in the BK channel α-subunit, underlies human epilepsy and paroxysmal movement disorder. Similarly, dentate granule neurons from mice lacking the β4 BK channel subunit show a GoF for BK channels that sharpen action potentials, thereby facilitating high-frequency firing and leading to temporal lobe seizures. Epilepsy was found to be caused by an increase in a potassium current rather than by a decrease, and BK channel-deficient mice have recently been shown to reduce EEG power.[124,125]

The Mechanism of CBD on Potassium Channels

Recent studies have shown that CBD affects *KCNT1* and BK channels to prevent seizures.[126–129] EIMFS patients were treated with CBD in add-on to their baseline AEDs and showed a notable reduction in seizure intensity with possible developmental progression.[126] This showed that CBD may be beneficial as an adjunctive medication in treating the *KCNT1*-related EIMFS patients.[126,130] Interestingly, pretreatment with Paxilline, a highly specific BK channel antagonist, blocked CBD anticonvulsant effects in mice submitted to the Pentylenetetrazol (PTZ) test.[127] Since BK channels activity is dependent of intracellular calcium levels and CBD is capable of interfering with calcium homeostasis mobilizing intracellular calcium stores in neuronal tissue,[128] it is possible that CBD action is in part due to the decrease in intracellular calcium levels that is likely mediated by BK channels during seizures.[129] However, CBD's side effect is also considered. CBD at a high concentration (10 μM) decreased inward late sodium, L-type calcium currents, hERG (human ether-a-go-go-related gene, same as KCNH2 gene encodes for a protein known as Kv11.1, the alpha subunit of a potassium ion channel) potassium channels, and delayed rectifier potassium current. Especially, hERG and potassium channel inhibition might have a role in the possible proarrhythmic adverse effects of cannabinoids, which are related to the sudden death of arrhythmias.[131]

Calcium Channels

Voltage-gated calcium channels (VGCCs) are widely expressed throughout the mammalian CNS, which are classified into low-voltage–activated (LVA; T type, $Ca_v3.1$,3.2, and 3.3) and high-voltage–activated (HVA) channels. The HVA family of calcium channels was further subclassified according to their conductance, kinetics, and sensitivity to pharmacological blocking agents, to L-type ($Ca_v1.1$, 1.2, 1.3, and 1.4), P/Q-type ($Ca_v2.1$), N-type ($Ca_v2.2$), and R-type ($Ca_v2.3$). HVA channels consist of a principal α1 subunit, which forms the channel pore, a β subunit, which is cytoplasmic, an extracellular α2δ subunit, which is attached to the membrane via a glycophosphatidylinositol (GPI) anchor, and possibly a γ subunit. LVA channels do not appear to associate with accessory subunits.[132]

Although mutations of SCN1A are the most frequent genetic cause of DS, it has been demonstrated that the Calcium Voltage-Gated Channel Auxiliary Subunit Beta 4 gene, encoding the beta4 subunit of voltage-dependent calcium channel, can affect the phenotypic expression of DS and the pharmacological response.[133] The modulation of Ca^{2+} currents may represent a mechanism contributing to the antiepileptic activity of both verapamil and levetiracetam in DS.[134] Calcium/calmodulin protein kinase II-mediated modulation of neuronal persistent sodium current impacts neuronal excitability $SCN2A^{Q54}$ in mice.[135] Calcium Voltage-Gated Channel Subunit Alpha1 G (*CACNA1G*) gene, encoding the Cav3.1 subunit of the T-type calcium channel family, is a genetic modifier of a mouse model of DS by mutation of VGSC *SCN1A+/-*, suggesting that Cav3.1 may be a potential molecular target for therapeutic intervention in DS patients.[136] Calcium Voltage-Gated Channel Subunit Alpha1 H (*CACNA1H*) gene encodes Cav3.2, a member of the T-type calcium channel family, did not alter survival in a DS mouse model. Further investigation on the role of Ca^{2+} currents in the pathophysiology and disease expression of DS may also contribute to the quest for new antiepileptic treatments. In vivo, 2-Photon calcium imaging of naturalistic seizures in awake, behaving mice were performed in the model of the prominent neurodevelopmental disorder of DS (*SCN1A+/-* mice), which provides Ca^{2+} transient information on the role of PV-INs in seizure initiation and propagation in DS and other epilepsies.[137]

The Mechanism of CBD on Calcium Channels

Compared with the studies on sodium and potassium channels, there are much more and detailed research on calcium channels. CBD anticonvulsant action through increasing intracellular calcium, T-type and L-type VGCC, endocannabinoid system (Cannabinoid receptor type 1 and 2: CB1 and CB2 receptors), G protein-coupled receptor 55 (GPR55) receptor, and transient receptor potential (TRP) channels[138] were summarized in the following:

Cannabinoids are highly lipophilic, allowing access to intracellular sites of action, and resulting in increases in calcium in a variety of cell types including hippocampal neurons. CBD actions on calcium homeostasis may provide a basis for CBD neuroprotective properties. Under control conditions, CBD induces increases in $[Ca^{2+}]i$; in contrast, in the presence of 4-AP (which induces seizure-like $[Ca^{2+}]i$ oscillations) or increased

extracellular K^+, CBD acts to reduce $[Ca^{2+}]i$ and epileptiform activity through an action on mitochondria Ca^{2+} stores.[139] This suggests that CBD-mediated Ca^{2+} regulation is bidirectional, depending on the excitability of cells. An increase in endocannabinoid signaling drive, associated with CBD modulation, is mediated by mechanisms of either an inhibition of its hydrolysis or an increase in calcium signaling, which are related to CBD's anticonvulsant effects.[17,139] There were significant increases in CACNA1H subunit and *CB2* gene expression in DS patients by CBD targeting analysis.[140] Cannabinoids are promising neuroprotective compounds; they close Ca^{2+} channels and prevent toxic intracellular Ca^{2+} buildup and reduce glutamate release.[141] Therefore, CBD produces biphasic changes in intracellular calcium levels via antagonism of the mitochondrial Voltage Dependent Anion Channel 1(VDAC1).[142]

CBD has a number of actions on ion channels which are targeted by other antiseizure drugs. CBD blocks human and native T-type and L-type voltage-gated calcium channels (VGCC).[143,144] CBD antagonizes T-type voltage-gated calcium channels,[145] which is a similar mechanism of action to some AEDs such as zonisamide and ethosuximide. Neuronal depolarization appears to be reduced by CBD's modulation of Ca^{2+} and Na^+ ion influx into the neuron by binding to human T-type voltage-gated Ca^{2+} channels and by melastatin- and vanilloid-type transient receptor potential membrane receptors.[146] When the postsynaptic neuron membrane is depolarized, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are produced from the postsynaptic membrane components and then released into the synaptic cleft, causing presynaptic CB1R receptor activation. This then results in a transient hyperpolarization of the presynaptic membrane through suppression in voltage-gated Ca^{2+} channels and activation of K1 channels. This transient hyperpolarization of the presynaptic neuron in turn suppresses further neurotransmitter release.[147] CBD is a partial negative allosteric modulator of CB1R whose anticonvulsant effect is independent of activation of the endocannabinoid system.

GPR55 was first identified as an orphan Class A G protein-coupled receptor (GPCR) enriched in the brain and was originally suggested as a novel cannabinoid receptor.[148,149] GPR55 has been shown to utilize Gq, G12, or G13 for signal transduction and the subsequent increased intracellular Ca^{2+} concentration through the release of inositol triphosphate (IP3)-gated intracellular Ca^{2+} stores and activation of RhoA and phospholipase C. GPR55 receptor expression is increased in the epileptic hippocampus.[150] The GPR55 receptor is a G13-protein–coupled receptor that is activated by endocannabinoids and antagonized by CBD.[151] Concerning GPR55 receptor signaling, its activation may lead to intracellular calcium increase, through the mobilization of both intracellular and extracellular calcium.[152] GPR55 antagonist pretreatment mimics CBD anticonvulsant effects, and no additional effect was observed when CBD was administered after the GPR55 antagonist, suggesting that CBD anticonvulsant effects may be, at least in part, due to the antagonism of GPR55 receptors.[16] Deletion of GPR55 in mice produces no conspicuous gross phenotypic, behavioral, or pathological changes, or obvious seizure susceptibility changes, which would be expected if inhibition of GPR55 is an antiseizure mechanism.[153] Several targets including the blockade of GPR55 and T-type VGCCs and stimulation of $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptors are considered.[154]

CBD has also been reported to be an agonist of transient receptor potential vanilloid 1 (TRPV1), a non-selective cation channel, which is expressed widely throughout the CNS and peripheral afferent fibers.[138,155] TRPV1 channel consists of 6 transmembrane domains with a non-selective hydrophobic pore between the fifth and sixth transmembrane domains that is responsive to chemical and physical stimuli. Activation of TRPV1 by the vanilloid capsaicin, noxious heat, low pH, various lipids, and other agents including phytocannabinoids such as CBD leads to Ca^{2+} influx through the channel. When activated, CB1 receptors inhibit synaptic transmission through action on voltage-gated calcium and potassium channels, which are known to modulate epileptiform and seizure activity.[156] Depending on the degree and duration of Ca^{2+} influx, the increase in intracellular Ca^{2+} can desensitize the channel, representing a protective negative feedback mechanism. TRP channels that are involved with the modulation of intracellular calcium are targeted by CBD.[157] CBD acts as an agonist at human TRP channels, specifically in the TRPV1 channel, which is in part responsible for calcium channel modulation.[158] TRPV1 expression is increased in human epilepsy and unsurprisingly plays a role in regulation of cortical excitability.[159,160] A recent research report showed that TRPV1 is a modest genetic modifier of spontaneous seizure severity but not a viable anticonvulsant drug target in the *Scn1a*$^{+/-}$ mouse model of DS.[161] Like GPR55, knockout of TRPV1 did not markedly impact chemoconvulsant seizures in neonatal mice.[162] TRPV1 knockout animal model test shows that CBD's anticonvulsive effects are through TRPV1 channels.[163] CBD activates vanilloid transient receptor potential channels TRPV1, 2, and 3 and the ankyrin subfamily member TRPA1 (prolonged exposure causes desensitization), in addition to antagonizing TRPM8 (melastatin-type).[164] Besides those mechanisms directly evaluated in *in vivo* animal models, there is *in vitro* data supporting those anticonvulsant mechanisms associated with intracellular calcium signaling through TRPV1 receptors.[165] Additionally, CBD restores changes in the hippocampal CA1 long-term potentiation in mice submitted to pilocarpine-induced SE through a mechanism dependent on $5\text{-}HT_{1A}$ and intracellular calcium stores, but independent of CB1 signaling.[166] CBD acts on TRPV channels as an agonist, especially on TRPV1 channel sub-type, inducing activation, dephosphorylation, and strong desensitization, which in turn decreases intracellular calcium levels and neuronal excitability,[138,167] thus accounting for both the CBD anti-nociceptive and anticonvulsant effects.

CBD has indirect effects by increasing endogenous anandamide expression. Anandamide affects excitability in neuronal networks by activating the TRP cation channel. CBD regulation of Ca^{2+} homeostasis via several mechanisms may contribute to these actions, particularly for partial or generalized seizures.[168] CBD inhibits TRPV1 signaling by a dual mechanism: the first by inhibiting the adenylyl cyclase–cAMP pathway, which is essential for maintaining TRPV1 sensitization. The second pathway likely involves calcineurin-mediated TRPV1 inhibition.[169] CBD anticonvulsant action is also related to affecting mitochondrial sodium/calcium exchanger. For example, CBD interaction with Na^{+}-Ca^{2+} exchanger (NCX), a mitochondrial sodium/calcium exchanger, supports a mechanism in which CBD mediates intracellular calcium levels. Through this mechanism, CBD prevents epileptic-like activity in cultured hippocampal neurons via the restoration of Ca^{2+} homeostasis.[170] In this context, another mechanism that could be involved in CBD and calcium modulation is the mitochondrial CB1 receptor (mtCB1).[171] Taken together, these findings suggest that calcium modulation could be involved, at least in part, on CBD anticonvulsant effects. While the precise mechanism of action of CBD in humans remains unknown, there exist at least 3 plausible molecular ionic channel targets discussed above in the anticonvulsant properties of CBD. Thus, there are other potential targets engaged by CBD beyond those described here. For example, CBD reduces neuronal excitability through functional antagonism of GPR55 receptors, desensitization of TRPV1 receptors and inhibition of adenosine transport.

HCN Channels

Hyperpolarization-activated cyclic nucleotide-gated ion (HCN) channels conduct the H-current (I_h) and are encoded by 4 genes (*HCN1, HCN2, HCN3*, and *HCN4*). Structurally similar to K^{+} voltage-gated channels, HCN channels are formed as a tetramer of subunits, each with a six-transmembrane domain topology, including a pore region that conducts ion flow, and intracellular amino and carboxyl termini.[172] HCN channels are cation permeable channels that are activated by hyperpolarization and deactivate upon depolarization of the membrane potential and modulate membrane resistance and resting potential; and can mediate pacemaker activity in some types of neurons due to their particular biophysical properties. HCN channels are voltage-gated ion channels that modulate excitability in several brain regions involved in the pathogenesis of epilepsy, including the hippocampus, neocortex, and thalamus. The HCN channel has emerged as a compelling new candidate channelopathy in epilepsy. Accumulated evidence shows that downregulation of I_h, the current generated by HCN channels, causes neuronal hyperexcitability,[173] and that genetic deletion of HCN1 channels, the main cortical and hippocampal subtype, accelerates the rate of epileptogenesis in acquired epilepsy models.[174] More recent evidence shows that mutations in *HCN1* underlie early life epileptic encephalopathy in some children with severe epilepsy and developmental delay.[175] Thus, *HCN1* channelopathy occurs in both human genetic epilepsy and animal models of acquired epilepsy. The epilepsy induced by chemoconvulsant-induced SE was associated with loss of *HCN1* channel expression that began within 1-hour post-SE and persisted into chronic epilepsy. HCN1 channels were acutely internalized from the surface membrane of hippocampal pyramidal dendrites within the first hour following SE, delayed loss of protein expression, and later downregulation of HCN1 mRNA expression. HCN1 channel surface expression is governed in a bidirectional fashion by protein kinase C (PKC) activity.[176] Therefore, H-currents appear to be dendritic AED targets.[177]

The Mechanism of CBD on HCN Channels

Although there are several studies on epileptogenesis that are related to HCN channels, there are very few studies on the effect of CBD on HCN channels in epilepsy. One report showed that cannabinoid-controlled learning and memory is through HCN channels.[178] Therefore, it is of interest to study whether CBD can ameliorate the cognitive impairment of DS via its interaction with the HCN channels.

Other Potential DS Treatments

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 such as DS. It is 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.[179] However, it is difficult to foresee which will be the potential drug developments for the treatment of DS. The following are potential DS treatments:

1. Food supply treatment: high-fat, very low-carbohydrate ketogenic diets, and milk whey help some patients with uncontrolled epilepsy by increasing serotonin (5-HT) level.[180]
2. Stem cell-derived interneuron transplants and mouse embryonic stem cells are used to treat a mouse model of DS.[38,181]
3. The endocannabinoid system and its modulators seem to play a key role in epilepsy treatment and pathophysiology. For example, reduced cannabinoid 2 receptor activity increases susceptibility to induced seizures in mice.[182] The α/β-hydrolase domain 6(ABHD6), a newly discovered enzyme, controls the amount of 2-arachidonoylglycerol (2-AG), the most abundant endocannabinoid (eCB) in the brain. The use of ABHD6 inhibitors decreases seizure incidence in several mouse models of epilepsy.[30]

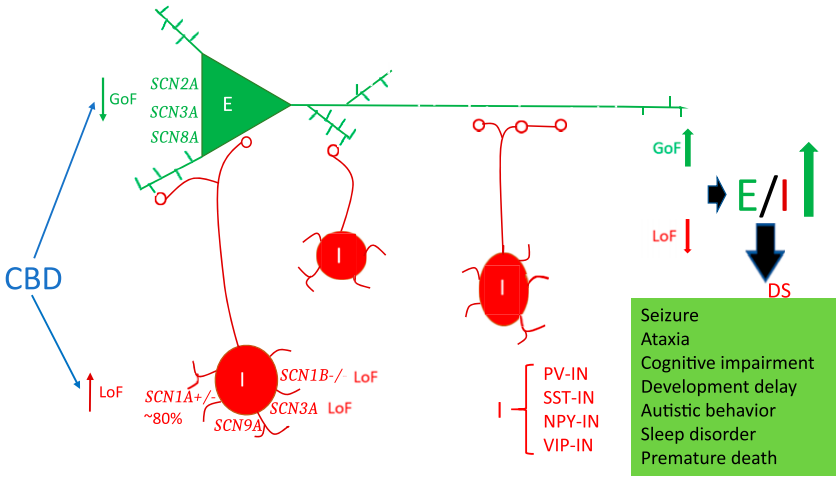

Figure 1. A diagram of an excitatory-inhibitory network in Dravet syndrome and CBD's effect on sodium channels of the network. The excitatory pyramidal neuron is illustrated in green and is surround by various types of inhibitory interneurons in red (PV-, SST-, NPY-, VIP-INs). The interneurons' synapses are on the cell body, dendrites, and axon of the pyramidal cell and regulate its activity. E: excitatory pyramidal neuron. I: inhibitory interneurons. E/I: excitatory-inhibitory ratio. CBD: cannabidiol. DS: Dravet syndrome. GoF: gain-of-function. LoF: Loss-of-function.↑: increase.↓: decrease. *SCN1A*: predominantly in inhibitory GABAergic interneurons, particularly in parvalbumin-positive interneurons (PV-INs). ~80%: *SCN1A* haploinsufficiency accounts for almost 80% cases of DS. *SCN2A and SCN3A*: especially expressed in excitatory neurons and glutamatergic neurons. PV: parvalbumin. SST: somatostatin. NPY: neuropeptide-Y. VIP: vasoactive intestinal peptide.

4. Antisense oligonucleotides increase SCN1A expression and reduce seizures and SUDEP incidence in a mouse model of DS.[183]
5. Potential use a microRNA-128 (miR-128)-based therapy for epilepsy, because microRNA-128 modulates the activity of signaling networks.[184]
6. Based on "interneuron hypothesis," the use of a Cre-dependent small hairpin RNA (shRNA) to rescue somatostatin-positive inhibitory interneurons (SST) excitability. Recently, selective activation of NaV1.1 by venom peptide Hm1a (Heteroscodratoxin-1) restores the function of inhibitory interneurons from DS mice without affecting the firing of excitatory neurons. Intracerebroventricular infusion of Hm1a rescues DS mice from seizures and premature death.[185]
7. Reversible acetylcholinesterase inhibitors (rAChels) (e.g., Huperzine A and donepezil) used in the treatment of dementia and Alzheimer's disease might be also therapeutic in the treatment of epilepsy. Recently, the Huperzine A provides robust and sustained protection against DS.[186]

Conclusions

Except for *SCN1A* gene encoding Nav1.1 channel in interneurons, other sodium subunits, potassium, calcium, and HCN channels in interneurons or excitatory neurons also contribute to the etiological channelopathy of DS. Essentially, CBD has shown neuroprotective effects on DS in preclinical experimental models and clinical data of DS. Concerning the mechanism of action, this review showed that CBD anticonvulsant effects might be due to its modulation to a great variety of ionic channels, including sodium channels, potassium channels, and calcium channels, which are known to play an important role in DS (see Figure 1.). These channels' loss of function or gain of function in interneurons or excitatory neurons causes imbalance of excitatory/inhibitory neurotransmission and CBD can restore the balance through potentiation of GABAergic/inhibitory or depression of excitatory neurotransmission, and calcium mobilization from BK channels, TRPV1, mitochondrial, and GPR55 receptors. Therefore, it is possible to assume that the CBD anticonvulsant activity might be due to its impact on multi-different ion channels underlying the channelopathy of DS. In addition, with further research on pharmacokinetic, pharmacodynamics, and molecular mechanisms of CBD in experimental models and clinical patients, the CBD action mechanisms will be better understood, and thus, it can provide further insight into the precise treatment of DS.

Acknowledgments

The authors thank Ms Callie Xu for her help in manuscript preparation.

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