Voltage-gated Sodium Channels and Blockers: An Overview and Where Will They Go?

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Summary: Voltage-gated sodium (Nav) channels are critical players in the generation and propagation of action potentials by triggering membrane depolarization. Mutations in Nav channels are associated with a variety of channelopathies, which makes them relevant targets for pharmaceutical intervention. So far, the cryoelectron microscopic structure of the human Nav1.2, Nav1.4, and Nav1.7 has been reported, which sheds light on the molecular basis of functional mechanism of Nav channels and provides a path toward structure-based drug discovery. In this review, we focus on the recent advances in the structure, molecular mechanism and modulation of Nav channels, and state updated sodium channel blockers for the treatment of pathophysiology disorders and briefly discuss where the blockers may be developed in the future.

Key words: voltage-gated sodium channels; blockers; Nav channel structures; channelopathies

Life did not come into existence until living organisms were enclosed by one or more membranes which cut them off from the chaotic world at a molecular level. Whereas, to respond to various cellular housekeeping reactions or stimuli given to the cell in the environment, the living cells developed the ability to overcome this barrier ingeniously by providing pathways to link the extracellular medium, cytoplasm and the contents of intracellular organelles. Indeed, transporting proteins embedded in membranes including pumps, channels and transporters are vitally important parts in these “pathways” to fulfill substances traveling across the membrane for the processes of energy transduction and cell excitability. Being the crucial components of living cells, ion channels regulate the passage of ions across the membranes and perform the primal functions to form the basis for the elaborate development of electrical properties that allow perception, thought, and motion. That is to say, studded with these ion channels in the membranes, the excitable cells could transduce electrical and chemical stimuli into currents of charged species.

In this review, we focus on voltage-gated sodium (Nav) channels, which selectively conduct sodium ions movement in response to variations of membrane potentials[1]. Defective Nav channels can give rise to channel malfunctions, leading to both neuronal hypo- and hyperexcitability, accordingly associated with acquired or inherited disorders (sodium channelopathies)[2, 3]. To date, more than 1000 point mutations of Nav channels have been detected in the central nervous system (CNS), the peripheral nervous systems (PNS), heart, skeletal muscles and cancer cells, which expanded our knowledge of the role of Nav channels in the wide spectrum of diseases such as epilepsy, migraine, pain, myotonic syndromes, diabetes, autism, cardiovascular diseases and so on[3–7]. For example, Nav1.7 subtype, preferentially expressed in the olfactory epithelium, sympathetic neurons, and dorsal root ganglion sensory neurons, plays a key role in pain transmission[8]. Dysfunction of Nav1.7 may predispose people to lots of disordered diseases like indifference to pain[9]. Therefore, Nav channels display major targets for various clinical therapeutics and natural toxins[10, 11].

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sodium channel from *Alpha proteobacterium HIMB114* (Rickettsiales sp. *HIMB114*) and NaVMs, a sodium channel from *Magnetococcus* sp. (strain MC-1) were reported. The eukaryotic Nav channels were structurally unrevealed until 2017. The resolutions of cryo-EM structures of a Nav channel from American cockroach (NavPaS) and a prototypal Nav channel from electric eel (EeNav1.4) in complex with the β1 subunit were determined to be 3.8 and 4.0 Å, respectively, displaying distinct conformations. In 2018, the insect NavPaS in complex with a gating modifier toxin Dc1a was identified, and scientists reported the cryo-EM structure of human Nav1.4-β1 complex at 3.2 Å. In 2019, cryo-EM structures of human Nav1.7 in complex with auxiliary subunits and animal toxins, and human Nav1.2-β2-μ-conotoxin KIIIA complex were reported. These structures could help gain insight into the molecular basis of functional mechanism of Nav channels and provide a path toward structure-based drug discovery.

I MOLECULAR STRUCTURE OF NAV CHANNELS

Nav channels are formed by a central α-subunit and one or more auxiliary β-subunits. As shown in fig. 1, the α-subunit constitutes two functionally entities, the ion-conducting pore domain and the voltage-sensing domains (VSDs). α-subunit contains four repeated units of homology (I–IV or D1–D4), each developed by α-helical transmembrane segments (S1–S6). In mammals, nine functional α-subunits (Nav1.1–Nav1.9) have been cloned. All α-subunits share nearly homologous structure topology, which makes it hard to design isofrom-selective blockers for particular Nav channel. The expression of Nav channel α-subunits is both cell and tissue specific. Nav1.1, Nav1.2, and Nav1.3 subunits are present in the CNS. Nav1.4 is the main subunit expressed in the skeletal muscle. Nav1.5 subunits are expressed primarily in cardiac muscle. Nav1.6 is located in both CNS and PNS. Proteins Nav1.7–Nav1.9 are mostly restricted to PNS. According to the sensitivity to the tetrodotoxin (TTX) isolated from the puffer fish, the nine α-subunit can be classified into two groups. Namely, Nav1.1, Nav1.2, Nav1.3, Nav1.4, Nav1.6, and Nav1.7 are TTX-sensitive as they are blocked by nanomol concentrations of TTX. Nevertheless, Nav1.5, Nav1.8 and Nav1.9 are TTX-resistant channels as they are inhibited by much higher TTX concentrations.

There are three functional states for Nav channels: a closed resting state, an activated state, and a fast inactivation state. Specifically, the structure basis for voltage sensitivity is constituted within S1–S4, which controls the gating, while S5–S6 segments enclose the pore domain and the sequences between them assemble the extracellular domains, the selectivity filter (SF), and the SF-supporting helices P1 and P2. Compared to calcium and potassium channels, Nav channels possess a highly asymmetric SF. Four amino acid residues including aspartate, glutamate, lysine, and alanine (DEKA) which are located in repeats I, II, III, and IV, respectively in the P region are crucial for sodium selectivity.

In particular, the fourth hydrophobic segment, S4, one in each domain of Nav channels heterotetramer, has a well-conserved motif of positively charged amino acids (arginine or lysine), which keeps the highly charged secondary structure poised to respond to the voltage changes by moving along the membranes in a stepwise manner. The mode of helical screw motion of S4 contributes to the dynamic process of the gate in the S6 segment. Movements of the charged residues are coupled to the opening of the pore domain upon membrane depolarization, and subsequently sodium ions travel across cell membrane. Mutagenesis studies carried out for Nav channels indicated that the shape and chemical complementarity between S4–S5 linker and the S6 C-term could work for the gating properties. Inactivation is an important functional feature of Nav channels for preventing a runaway of potential stability. There is evidence indicating that the conserved intracellular linker between domain III and domain IV is critical for fast inactivation. A set of hydrophobic amino acids (isoleucine, phenylalanine, methionine and threonine) called IFMT motif is involved in rapid inactivation by closing the entrance of the pore. Many details on the binding site for the inactivation are still controversial. Nonetheless, it is commonly accepted that the Nav...
channels inactivation comes from its specific pore sequences. With evolution and structural studies carried out, this distinctiveness may endow it with the exclusive privilege as an excellent drug target.

β-subunits are transmembrane proteins, and each one is composed of a single α-helix stretching across the membrane. With a large extracellular N terminus, Nav channels β-subunit displays an immunoglobulin outer segment to bind external proteins and modulates membrane trafficking, channel gating kinetics, and voltage dependence.

In 2017, the first near-atomic resolution structure of eukaryotic Nav channel, NavPaS, reveals that its intracellular gate is firmly closed and the side walls are sealed without fenestrations, reminiscent of what may not represent an inactivated state\(^{[16]}\) (fig. 2). Nevertheless, the VSDs show “up” conformations but are distinct from entirely activated conformations. Meanwhile, the intact III-IV linker is sequestered by the globular C-terminal domain (CTD), which is unlikely to be able to conduct its inactivating function. In addition, the IFM motif that is crucial for fast inactivation is replaced by Ala/Thr/Asp in NavPaS. Nonetheless, the near-atomic resolution structure of a single chain eukaryotic Nav channel was established as an important foundation for investigating the function-disease mechanisms of Nav channels and for structure-guided drug development. In other words, the structure opens a new chapter for mechanistic investigation of Nav channels and drug discovery for Nav channelopathies. In the same year, the structure of EeNav1.4 was reported (fig. 3). In contrast with NavPaS, EeNav1.4 exhibits features as follows. (i) The intracellular gate is kept open by a digitonin-like molecule. (ii) Whereas side groups could not be assigned for VSDs in repeats I and II, the resolved VSDIII and VSDIV both exhibit “up” conformations. (iii) The IFM motif is positioned between the inner S6 segments and the outer S4-S5 linkers in repeats III and IV\(^{[19]}\). It should be noted that electrophysiological states of the two sodium channels are yet to be defined for their structures in the absence of other reference structures.

Almost all venoms from nature contain toxins that regulate the activity of Nav channels in order to paralyze prey or predators. Neurotoxins like TTX and saxitoxin (STX) function as pore blockers, modulate the channel in a particular stage of the gating cycle through interacting with one or more VSDs\(^{[32]}\). Scientists presented structures of the insect Nav channel NavPaS bound to a gating modifier toxin Dc1a at 2.8 angstrom-resolution and in the presence of TTX or STX at 2.6-Å and 3.2-Å resolution, respectively, which elucidated the molecular basis for the insect selectivity of Dc1a and the subtype-specific binding of TTX or STX to Nav channels\(^{[18]}\) (fig. 4). In the structure of NavPaS-
Dc1a complex, VSD II showed an important role in binding the gating modifier toxin (GMT). In addition, it displays the interaction between the toxin, the S5-III pore-domain and the extracellular dome above the pore.

To further study the structure-function relationship of Nav channel, scientists reported the cryo-EM structure of human Nav1.4-β1 complex at 3.2Å[19] (fig. 5). This accurate structure provides important insight into the molecular basis for series of process of sodium function including Na⁺ permeation, electromechanical coupling, asynchronous activation, and fast inactivation of the four repeats, which provided an accurate template to map mutations linked with diseases such as myotonia and periodic paralysis. In this structure, Voltage sensing involves 4–6 Arg/Lys residues on S4 of the VSD. This helix moves “up” due to the changes of the membrane potential, which opens the channel in the end. All four VSDs exhibit up conformations. IFM motif between repeats III and IV inserts into the hydrophobic cavity which is enclosed by S4-S5 segments and the S6 in repeats III and IV. This allosteric blocking mechanism for fast inactivation is verified by the interactions between functional residues and disease mutations.

Studies revealed that the four β subunits (β1 to β4) affect the properties of Nav1.7 channel. β1 and β2 are commonly co-expressed with the α subunit of Nav1.7 for biophysical characterization[33]. Recently, the cryo-EM structures of the human Nav1.7-β1-β2 complex bound to two combinations of gating modifier toxins (GMTs) and pore blockers, saxitoxin with huwentoxin-IV (HWTX-IV) and tetrodotoxin (ProTx-II) with protoxin-II, both determined at 3.2 angstroms[20] (fig. 6). In the inactivated conformation, the fast-inactivation IlePhe-Met motif in III-IV linker inserts into the cavity between the S4-S5 restriction ring and the S6 helical bundle, providing support for the structural basis of “allosteric blocking” mechanism for fast inactivation. The binding mode for these two toxins by Nav1.7 is different from that for Dc1a by NavPaS. Dc1a projects into the extracellular cavity that is enclosed by the segments in VSD II and the PD. Indeed, the pore domain provides the support to anchor Dc1a, and then traps VSD II in a specific conformational state. In contrast, HWTX-IV and ProTx-II bind to the region that links S3 and S4 in VSD II. The distinction of binding mode provides the support to anchor the GMTs, and then locks the associated VSDs in functional states to modulate gating. Xu et al visualized ProTx2 in complex with VSD2 of Nav1.7 by X-ray crystallography and cryoelectron microscopy. ProTx2 was found to be trapped in activated and deactivated states of VSD2, which revealed a remarkable ~10 Å
translation of the S4 helix, and provided a structural framework for activation gating\cite{34}.

Meanwhile, the published cryo-EM structure of human Nav1.2 bound to the m-conotoxin K\textsubscript{IIIA} (a peptidic pore blocker) in the presence of \(\beta 2\) is at 3.0 angstroms\cite{21} (fig. 7). The immunoglobulin domain in \(\beta 2\) interacts with the shoulder of pore domain by a disulfide bond. K\textsubscript{IIIA} interacts with the specific residues in repeats 1 to III, revealing a molecular basis for K\textsubscript{IIIA} specificity. To date, Structural determination of the insect NavPaS in complex with Dc1a, the human Nav1.4-\(\beta 1\) complex, the human Nav1.7 with two tarantula toxins, HWTX-IV and ProTx-II, and the human Nav1.2 with K\textsubscript{IIIA} in the presence of \(\beta 2\) will provide a path toward structure-based drug discovery.

2 CHANNELOPATHIES RELATED TO NAV CHANNELS

2.1 Pain

Pain, a physiological sensation, is perceived by organisms as it alerts organisms to dangers around the corner. Chronic neuropathic pain is associated with severe painful sensation which may derive from innocuous or acute pain stimuli, and it is often characterized by long duration and no particular trigger. Despite that there are numerous options for the treatment of chronic pain, it is still intractable to achieve the management of these syndromes. Discovery and development of new therapies for neuropathic pain represents a serious challenge. In fact, to date, it has been established that sodium channels are essential for the transition of pain sensation. In particular, at least three subtypes presented in human dorsal root ganglion (DRG) neurons, Nav1.7, Nav1.8 and Nav1.9 have been identified as the key players in transportation of pain signals\cite{35-39}. Nav1.7 channels activate and inactivate rapidly. They, however, recover slowly from inactivation. Nav1.7 is considered a predominant Nav channel to produce substantial currents responding to depolarizations, which lead to neuronal firing produced by subthreshold stimuli.

Levels of activated p38 mitogen-activated protein kinase (MAPK) and signal-regulated kinase (ERK1/2) were increased in blind-ending axons of painful human neumomas with upregulated Nav1.7, suggesting that modulation of the Nav1.7 channel by MAPKs may be one of the mechanisms contributing to the development of ectopic discharges in nociceptors and neuroma associated pain in humans\cite{40, 41}. Genetic studies on patients with pain disorders revealed missense mutations in SCN9A encoding Nav1.7. Biophysical studies demonstrated that these mutations modulate Nav channel activation and/or inactivation properties and lead to hyperexcitability in DRG neurons\cite{39}. Gain-of-function mutations of Nav1.7 have been identified in patients with inherited erythromelalgia\cite{42}, which is an autosomal dominant condition characterized by erythema of the extremities and burning pain, as well as in small fiber neuropathy known as painful neuropathy\cite{43}, composed of small fiber related symptoms, including autonomic dysfunction, pain, loss of pinprick and allodynia, or hyperalgesia. However, loss-of-function mutations in Nav1.7 affected individuals unable to feel pain in the families with congenital insensitivity to pain (CIP).

Compared to Nav1.7 channels, Nav1.8 channels activate at more depolarized membrane potentials, and mediate the majority of the inward sodium currents during the depolarization of neuronal action potentials that are important for the transmission of action potentials as well as repetitive firing. Heterozygous gain-of-function mutations in SCN10A encoding Nav1.8 enable hyperexcitability and abnormal firing of DRG neurons, which is also associated with painful small-fiber neuropathies\cite{44}. Mounting evidence indicates the nociceptive neuron function of Nav1.8. Similarly, mutations of Nav1.9 channels encoded by the gene SCN11A modulate Nav channels gating properties, and lead to depolarized potentials of membrane, likely resulting in abnormal excitability of DRG neurons\cite{45}.

2.2 Migraine

Familial hemiplegic migraine type 3 (FHM3) is a severe autosomal dominant inherited subtype of FHM characterized by hemiparesis and visual aura during attacks\cite{46}. Evidence from studies in patients has indicated that cortical spreading depression (CSD) is a torpidly propagating wave of neuronal and glial depolarization with an increase in potassium ion concentrations, stimulating trigeminovascular system from the meninges and activating downstream pain pathways, and possibly leading to headache. FHM3 is associated with dysfunction of Nav1.1, which varies from gain of function to complete loss of function\cite{47}. The pathogenetic mechanisms have not been completely
illuminated yet. A current hypothesis declares that abnormal voltage-gated sodium channels bring about neuronal hyperexcitability, increased release of neurotransmitter, and accumulated concentration of extracellular potassium, and ultimately lead to migraine.

2.3 Epilepsy

Epilepsy is a severe acquired or inherited disease characterized by abnormalities in neuronal activity, particularly, sustained firing of sodium-dependent action potentials due to a slow depolarized potential. Missense mutations in sodium channels encoding specific subunits including SCN1A (encoding the Nav1.1 α-subunit), SCN2A (encoding the Nav1.2 α-subunit), SCN9A (encoding the Nav1.7 α-subunit), SCN1B (encoding β1-subunit), and SCN3A (encoding the Nav1.3 α-subunit) are responsible for genetic epilepsy syndromes along with a wide range of severity, which include generalized epilepsy with febrile seizures plus (GEFS+), benign familial neonatal-infantile seizures, and Dravet syndrome. Missense mutations impair the excitability of inhibitory interneurons, leading to hyperexcitability and various epileptic seizures[49]. In particular, Nav1.1 channel is expressed in GABAergic interneurons synthesizing and releasing γ-amino butyric acid (GABA, the major inhibitory neurotransmitter in the brain). Interneurons are important parts to regulate neuronal network excitability and synchronization of neuronal activity. Currently, the mechanism of epilepsy caused by the mutations is unclear, but it is thought to be related to the impaired generation of the current and action potential in GABAergic inhibitory neuronal Na+ channels[50].

2.4 Cardiovascular Diseases

The most common cardiac arrhythmogenic diseases associated with Na+ channelopathies are long QT syndrome (LQT3) and Brugada syndromes (BrSs)[51]. The significance of Nav channels in the normal cardiac electrical function has been underlined by the discovery of cardiovascular diseases linked with mutations in SCN5A, the gene encoding the Nav1.5 subunits[52]. Most of these mutations destroy fast sodium channel inactivation, and impair the channel ability to close completely, thereby generating persistent sodium currents and prolonging the ventricular action potential[53]. In contrast, mutations that reduce INa decrease cardiac excitability, reduce velocity of electrical conduction, and in extreme cases, lead to BrSs, sick sinus syndrome, or combinations thereof. Genetic studies have also showed that SCN10A variants encoding Nav1.8 subunit could disturb Brugada syndrome and cardiac conduction, which demonstrated that the emergence of SCN10A may be act as a cardiac arrhythmia susceptibility gene[54].

2.5 Neurodegenerative Diseases

Nav channels have been suggested to play a key role in processes involved in CNS disorders such as amyotrophic lateral sclerosis (ALS), the classical neurodegenerative disease Alzheimer’s disease (AD), multiple sclerosis (MS), Parkinson’s disease (PD), and Huntington disease (HD). Patients with MS show both negative (paresis, hyposthesia, and ataxia) and positive (pain and dysesthesia) symptoms. In addition, therapeutic approaches based on neuroprotection have been envisaged since decades to limit the neuronal damage consequent to severe insults, like traumatic brain injury (TBI) and intracerebral haemorrhage (ICH). Great efforts have being done to find methods effective on the progression of neurodegenerative diseases or secondary injuries. It was assumed that in those cases, featured by a reduced energy supply, leading to impaired action of Na+/K+-ATP-dependent pumps and depolarization of membranes, there may exist a greater persistent sodium ion conductance, most likely associated with Nav1.6. Sodium ions overload in the axon, accompanied with potassium ions efflux, may cause the accumulation of calcium ions via reversal of the Na+/Ca+ exchanger, and trigger a succession of pathogenic cascade, which ends with neuronal loss. An persistent increase in Na+ conductance, is probably to contribute to peripheral hyperexcitability in ALS, which leads to the classic symptoms of fasciculations and cramps, possibly resulting in the release of abnormal glutamate, which may play an important role in neurodegeneration[55].

2.6 Psychiatric Disorders

Mutated Nav channels have been found in patients with certain psychiatric conditions, although these studies need to be confirmed by the analysis of a higher quantity of individuals. Data suggested that mutations in SCN8A may lead to cognitive deficits and SCN8A may be a potential susceptibility gene for Bipolar disorder[56]. In addition, one study on subjects with Dravet syndrome, a severe epilepsy often caused by mutations in SCN1A gene indicated that mutations in SCN1A gene can be responsible for both epilepsy and progressive severe mental impairment[57].

2.7 Cancer

Analyses of various tumor tissues revealed that their intrinsic concentration of sodium ions is significantly higher than that of normal tissues and is closely related to the formation of tumors[58]. Nav channels, play an essential control function in cellular processes, including metastasis and angiogenesis, by regulating cell motility as well as the secretion of proteolytic enzymes. For example, the dysfunction of Nav1.5 channels and β subunits can make cancer cells highly invasive. Over the past years, Nav channel inhibitors have displayed beneficial effects in diverse types of cancer, such as prostate cancer, lymphoma, mesothelioma and cervical cancer, and have been involved in regulation of carcinogenic process,
including cell proliferation, invasiveness, and so on. However, the molecular mechanism involved in the regulation of Nav channels activity is still unclear. Currently, there are some hypotheses. The Na\(^+\) influx activates the sodium-hydrogen antiporter (NHE1), leading to a low intracellular pH, which enhances the activity of cathepsins, and therefore degrades the extracellular matrix\(^{[18],\ 60}\). After VGSC activation, mitochondria rapidly uptake Na\(^+\) and release Ca\(^{2+}\) into cytosol\(^{[61]}\), then promote the formation of invasive podosomes and enhance tumor cell invasion. The β subunit changes the morphology and movement of tumor cells by adhesion mechanisms that are related to fyn kinase and Na\(^+\) current\(^{[58]}\).

### 3 ADVANCES IN THE DEVELOPMENT OF NAV CHANNEL BLOCKERS FOR THE TREATMENT OF CHANNELOPATHIES

#### 3.1 As Pain-Relieving Drugs

If the root cause of neuropathic pain is DRG hyperexcitability derived from enhanced NaV function, it stands to reason that selective NaV blockers should be anti-hyperalgesic. Experimental evidence indicated that local anesthetic (LA), class I cardiac antiarrhythmics, and antidepressants possess properties of releasing neuropathic pain by modulating Nav channels. Nevertheless, their actual efficacy to treat chronic pain is under investigation due to their off-target adverse effects such as cardiotoxicity, ataxia, confusion and sedation, which therefore narrow their therapeutic margin. Small molecules ICA-121431 and PF-04856264 (inhibiting Nav1.7 with an IC\(50\) of 28 nmol/L) bind to Nav voltage sensor of DIV\(^{[62]}\) and would certainly address the role of this novel binding site for pain control. Many companies are developing the second generation of molecules which are isoform-selective NaV blockers. For example, CVN1014802 (now in Phase III clinical trials for treatment of trigeminal neuralgia\(^{[63]}\)) from Convergence Pharmaceuticals/Biogen Idec, and PF-05089771 from Pfizer are the most advanced clinical NaV1.7 blockers being tested in patients. Mutagenesis studies revealed that the compounds bind to the residues of S2 and S3 in the voltage-sensing domain IV, which has also been verified by the crystal structure of the Nav1.7 voltage-sensing domain IV, an aryl sulfonamide complex\(^{[64]}\). Data for orally administered XEN402 targeting NaV1.7 have been reported in the peer-reviewed literature and the authors reported that XEN402 (i) significantly reduced the ability to induce pain from heat or exercise, as well as the amount of pain after induction\(^{[65]}\), (ii) increased the time to reach maximal pain induction. Xenon developed XEN403 (a selective Nav1.7 blocker) as a backup candidate to XEN402, for the potential treatment of pain\(^{[66]}\).

### 3.2 Nav Channel Blockers for Migraine

Antiepileptic drugs (AEDs), originally approved for the treatment of epilepsy, are one of the major categories of conventional drugs as sodium-channel blockers to treat migraine. Recent evidence from clinical trials suggests that some sodium channels blockers (for example, topiramate, lamotrigine and carbamazepine) seem to be efficacious for migraine therapy, reducing aura as well as migraine attacks. In particular, topiramate presents high efficacy in reducing the intensity, duration and frequency of migraine. Moreover, several studies showed both good tolerability and high efficacy for valproic acid\(^{[75]}\). Nevertheless, the detailed mechanism of action of these AEDs for the treatment of migraine need to be further explored.

#### 3.3 Nav Channel Blockers for Epilepsy

Nav channels initiate action potentials in brain neurons, and sodium channel blockers are used in trials on GDC-0276 and GDC-0310 from Genentech, Nav1.7-selective inhibitors for the treatment of pain\(^{[67]}\), have been completed in 2017. PF-04531083 from Pfizer is designed as NaV1.8 blockers\(^{[68]}\), which is being tested into Phase II clinical trials. Moreover, several inhibitors including DSP-2230 and AZD-3161 have been advanced to clinical trials. In 2017, AM-0466 was identified as a Nav1.7-selective inhibitor, and showed a favorable pharmacokinetic profile in a licking time in a capsaicin-induced nociception model of pain\(^{[69]}\). In 2018, GX-201 and GX-585 was identified as Nav1.7-selective inhibitors with the acylsulfonamide scaffold, and showed analgesic activity in inflammatory and neuropathic pain models\(^{[70]}\). Unfortunately, no selective blockers of NaV1.9 have been identified thus far. A feasible mechanism to regulate Nav channels in a selective pattern was exemplified by natural neurotoxins from the venom of snakes, scorpions and other animals\(^{[71],\ 72]}\). These peptides have a variety of binding sites, particularly, the voltage sensors, which display less degree of observed sequence similarity than the pore-forming region. For example, D-conotoxins target DIV S3–S4 and slower inactivation, and scorpion α-toxins could bind to DII-VSD\(^{[73]}\). In addition, μ-SLPTX-Sm6a, a peptide toxin from the venom of centipede, shows selectivity inhibition to Nav1.7 by transition from its activation to depolarized potentials. Notably, the modulation capability of HWTX-IV toxin is tailored on stabilizing the rest state of D II-VSD of Nav1.7, and blocking the channel\(^{[74]}\). μ-Conotoxin KIII A, a peptide toxin from Cone snail, could bind to DI–DIV (pore region); μ-Conotoxin MrVIA, a peptide toxin from Cone snail, could bind to DII and E2. The crystallographic study of the toxin-channel interaction could contribute to mechanistic insights into the modulation of Nav1.7 by blockers, and hold promise to make Nav1.7 as an irresistible target for drug design.
therapy of epilepsy. Anticonvulsants, such as phenytoin (PHT) and carbamazepine (CBZ), are broad-spectrum antiepileptic drugs for the treatment of seizures with a frequency-dependent and voltage-dependent manner, and have an exceptional range of anticonvulsant efficacy against both partial and generalized tonic-clonic seizures. And they presumably act by attenuating sustained repetitive firing and voltage-gated sodium currents. Valproate and topiramate likely also act as sodium channel blockers as part of their therapeutic actions. Evidence has revealed that lacosamide represents an effective treatment for seizures by enhancing channel slow inactivation, and therefore, inhibiting Nav channels. PHT and CBZ possess prominent ability of voltage-dependent blocking due to their preferential binding to the inactivated states of Nav channel. The narrow therapeutic range of these drugs have raised serious concerns for the treatment of epilepsy. Shaheen et al reported a potential sodium channel blocker, NSC403438, as a better candidate than PHT and CBZ. It should be noted that sodium channel-blocking antiepileptic drugs are used broadly in the treatment of both generalized and partial seizures arising from different causes. But they are not effective in the treatment of absence seizures, driven by rhythmic firing in thalamic neurons that generate sleep rhythms.

3.4 Nav Channel Blockers for Cardiovascular Diseases

Class I antiarrhythmics, such as quinidine, procainamide, and disopyramide (class Ia agents) as well as mexiletine, lidocaine, phenytoin and tocainide (class Ib agents) and flecaïnine and propafenone (class Ic agents) play essential roles in the therapy of cardiac arrhythmias with other side effects due to their nonselective action. Ranolazine, approved by the FDA in the USA, is useful for the therapy of chronic angina pectoris in the patients with ischemic heart disease. However, ranolazine is only weakly against the persistent sodium current and poorly selective versus several potassium channels. For these reasons, it is reasonable to suppose a combination of effects for this drug. GS-458967 and F15845 are the selective inhibitors of cardiac sodium current. Moreover, F15845 plays a role in long- and short-term cardioprotection after myocardial infarction. Meanwhile, F15845 does not affect peak sodium current in normal polarized myocytes at pharmacologically active doses. This feature distinguishes this compound from the commercially available NaV1.5 blockers (as class I antiarrhythmics).

3.5 Nav Channel Blockers for Neurodegenerative Diseases

Among compounds so far investigated, AEDs have displayed effective compete against cell apoptosis or necrosis in vitro and in vivo models of specific neurological diseases. Concerning the mechanism of activity, AED drugs counteract the abnormal neuronal excitability via modulating the action of ion channels, receptors and signaling pathways. Among them, sodium channels are the main targets of AEDs in CNS. The increasing data have been reported that the damaged cognitive performance was related to reduced expression of Nav1.1 subtype in heterozygous SCN1A ± mice. Meanwhile, reduced short-term memory was observed in otherwise healthy humans who carried SCN1A mutations, suggesting that a potential relevance between reduced expression of Nav1.1 and cognitive deficits. Therefore, Nav1.1 activators have potential for the symptomatic treatment of cognitive dysfunctions such as Alzheimer’s disease and schizophrenia. In addition, Nav1.2, Nav1.6, and Nav1.8 seem to be associated with pathophysiology of MS, probably as a result of disregulated immune responses. Nav1.2 and Nav1.6 appear to play a key role in the restoration to neuronal conduction after axonal degeneration.

The upregulation of Nav1.8 was observed in a mouse model of MS, in which cerebellar dysfunction also emerged. In addition, MS deficits in the mouse model could be reversed partially with A803467 (Nav1.8 blocker). Lidocaine and mexiletine, voltage-gated sodium channels blockers, have the ability to block the symptoms of MS, therefore acting selectively on fibers which mediate the positive symptoms. Although the mechanism of selective degeneration of motor neurons has not been entirely understood, several hypotheses were proposed that altered functionality of Nav channels may be the chief criminal. Evidence showed that riluzole, a drug to treat ALS, tended to block TTX sensitive Nav channels. Meanwhile, riluzole also shows a neuroprotective action in the rat model of Parkinson’s disease. Remacemide and safinamide are also sodium channel blockers for the treatment of this disease in clinical trials. Experiment data have showed that the β-subunits of Nav channels are processed by BACE1 and γ-secretase, which may provide new view for understanding of Alzheimer’s disease.

3.6 Nav Channel Blockers for Psychiatric Disorders

Nav channel blockers have played a major role in the treatment of psychiatric disorders, for example, borderline personality disorder (BPD) and bipolar depression (BD). Compounds belong to the anticonvulsant class have displayed beneficial effects in therapeutic approaches to psychiatric disorders such as schizophrenia. Nevertheless, it is difficult to believe that blocking sodium channels expressed in the limbic system is the only mechanism of action of the drugs, and more studies for candidate mechanisms need to be carried out. Experimental data suggested that mutations in SCN8A may cause cognitive deficits. Carbamazepine and lamotrigine are the only FDA approved drug molecules targeting voltage gated sodium channels for the treatment of psychiatric disorders.
Carbamazepine was approved for the treatment of depression and acute mania in 2004. Lamotrigine has been approved for the treatment of BPD. Other sodium channel blockers like topiramate, oxcarbazepine, and zonisamide possess the capacity of stabilizing mood in schizoaffective and bipolar disorders. NW-3509, a new sodium channel blocker, showed efficacy in a broad spectrum of depression, psychosis, mania, and aggressiveness. After combination with antipsychotics, NW-3509 may improve their efficacy with a decrease of their dosage and side effects.

3.7 Nav Channel Blockers for Cancer

The main strategy for cancer therapy is to use sodium channel interfering drugs, especially acting on the NaV1.5 or NaV1.7 channel[80]. Sodium channel inhibitors proven to suppress tumor progression are the neurotoxin TTX, which inhibits cell invasion in breast in vitro. Phenytoin and a-hydroxy-aphenylamides were proven useful to treat prostate cancer. In addition, riluzole, ranolazine, phenytoin, lidocaine, and carbamazepine have shown cell metastatic blocking behavior in \textit{in-vitro} models. However, it is unrealistic to use these existing sodium channel blocker to treat cancer, because many of them, for example, anticonvulsants or antidepressants, could travel across the blood-brain barrier and function on isofoms without selectivity, thus causing many side effects.

4 CONCLUSION

In short, mutations in genes encoding for different Nav channels have been approved to play a key role in pathological disorders. Moreover, phylogenetic analyses have ensured the relationship between these channels with Nav channels from an evolutionary perspective. However, Nav channel blockers have typically showed poor selectivity to specific isofoms due to a high degree of sequence similarity between the subtypes, thus raising potential risks of many side effects. Nevertheless, Nav channels endowed with multifarious therapeutic potential could serve as interesting targets for drug discovery in the emerging therapeutic fields. To date, structures of the insect NavPaS in complex with Dc1a, the human Nav1.4-β1 complex, the human Nav1.7 with two tarantula toxins, HWTX-IV and ProTx-II, and the human Nav1.2 with KIIIA in the presence of β2 have been reported. These structures help understand their pharmacological profiles, as well as give insight into the molecular basis of sodium functional mechanism for ion permeation and provide a path toward structure-based drug discovery. In spite of these remarkable studies holding promise to structure-based drug design, it still represents great challenges due to the transitions between working states of voltage-dependent sodium channels entailing intricate conformational changes and reconfiguration of interactions between structural elements by direct or allosteric effects. Based on this, great efforts need to be conducted to interpret the interior relations of Nav mutations and corresponding structures based on the published specific structures for the treatment of channelopathies.

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Conflict of Interest Statement

The authors claim that the researchers in this study have no conflict of interest.

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