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Tuning Sodium Channel Blockers to the Near-Atomic Level

Dual-Pocket Inhibition of Na_v Channels by the Antiepileptic Drug Lamotrigine

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Voltage-gated sodium (Nav) channels govern membrane excitability, thus setting the foundation for various physiological and neuronal processes. Nav channels serve as the primary targets for several classes of widely used and investigational drugs, including local anesthetics, antiepileptic drugs, antiarrhythmics, and analgesics. In this study, we present cryogenic electron microscopy (cryo-EM) structures of human Nav1.7 bound to two clinical drugs, riluzole (RLZ) and lamotrigine (LTG), at resolutions of 2.9 Å and 2.7 Å, respectively. A 3D EM reconstruction of ligand-free Nav1.7 was also obtained at 2.1 Å resolution. RLZ resides in the central cavity of the pore domain and is coordinated by residues from repeats III and IV. Whereas one LTG molecule also binds to the central cavity, the other is found beneath the intracellular gate, known as site BIG. Therefore, LTG, similar to lacosamide and cannabidiol, blocks Nav channels via a dual-pocket mechanism. These structures, complemented with docking and mutational analyses, also explain the structure-activity relationships of the LTG-related linear 6,6 series that have been developed for improved efficacy and subtype specificity on different Nav channels. Our findings reveal the molecular basis for these drugs' mechanism of action and will aid the development of novel antiepileptic and pain-relieving drugs.

Commentary

Since the introduction of phenytoin as an anti-seizure medication (ASM) in 1938, blockers of voltage-gated sodium channels (Na_v) have been a mainstay of epilepsy treatments.[1] Some ASMs, such as phenytoin, carbamazepine, oxcarbazepine, and lacosamide, are presumed to exert their anti-seizure effects primarily by modulating either the fast or slow inactivation state of the channel. Others, for example, cannabidiol and cenobamate, have been shown to have additional mechanisms of anticonvulsant action.[2] Generally, "traditional" sodium channel blockers have similar efficacy in controlling focal seizures with some being additionally used for treating generalized epilepsies.[1] The scope of activity has not been fully established for the newer sodium blockers, but their adverse effect profiles might differ from those of older drugs (eg, with regard to sleepiness[3] or induction of drug-metabolizing enzymes).

To dissect the differences between sodium-blocking ASMs, we need to better understand how Na_v channels contribute to seizures, what subtype(s) of channels they bind, and where within the channel the binding site is. In humans, 9 Na_v channels govern the firing of electrical signals in neurons and muscles. The channels consist of pore-forming α subunits with an ion-selectivity filter, 4 voltage-sensing domains, and auxiliary β-subunits that regulate channel function and localization.[4] Over the past few years, high-resolution structures of eukaryotic Na_v channels obtained using cryo-EM expanded our understanding of how channel blockers exert their effects at the near-atomic level.[4] Recently, the group of Nieng Yan demonstrated that within $Na_v1.7$ channels, carbamazepine and lacosamide occupy the same site beneath the intracellular gate and tighten the gate in a similar manner.[4] This agreed with the already-known shared binding site of phenytoin, lamotrigine, carbamazepine, local anesthetics, and antiarrhythmic drugs.[5] Yet the high resolution allowed identifying a novel binding site for a second lacosamide molecule, the selectivity filter. The same group additionally discovered 2 binding sites for cannabidiol and demonstrated that neither of the 2 bound cannabidiol molecules is in a position to physically occlude the pore, suggesting allosteric inhibition.[6]

The featured article by Huang et al[7] takes this field one step forward with new information on lamotrigine binding sites. Using $Na_v1.7$ as a model sodium channel, dual-pocket inhibition was discovered also for lamotrigine. One lamotrigine binding pocket was within the central cavity of pore domain, in common with those of multiple other drugs. The other was the binding site for carbamazepine and lacosamide.[4] Thus, dual-pocket inhibition emerges as a common mechanism among Na_v-targeting ASMs with diverse molecular structures.

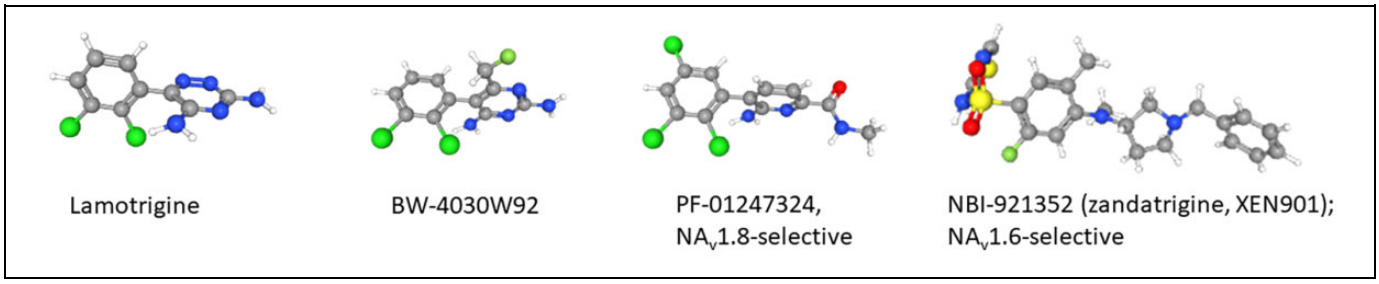

Figure 1. Structures of several Na_v selective and nonselective blockers. Source: PubChem.

The investigators further demonstrated *in silico* that a fluoride-containing group of the lamotrigine analog BW-4030W92 (Figure 1) occupied an additional hydrophobic pocket within the channel, in line with the higher potency of this analog. Another analog, PF-01247324 (Figure 1) docked into the corresponding locus within another sodium channel, $Na_v1.8$. The same analog was spatially incompatible with the equivalent pocket in the $Na_v1.7$ channel due to a difference between the channels of a single amino acid.

The dual-pocket inhibition unveiled in the current study is not yet sufficient for deciphering the differences in clinical activity between lamotrigine and other Na_v-blocking ASMs (eg, in sleepiness). Yet it adds to the increasingly growing pool of ASMs, channel-selective and nonselective ASM analogs, and other drugs, that is necessary for AI-assisted development of newer-generation ASMs. Several examples of potential improvements in Na_v blocker characteristics are described below.

Brain Selectivity

Na_v channels share a high degree of sequence similarity and many sodium channel blockers are not subtype-selective.[5] The genes most frequently associated with inherited epilepsies are those encoding $Na_V1.1$ and $Na_V1.2$ that are primarily expressed in the brain,[5] but a drug that docks into pockets of these proteins might also inhibit the cardiac $Na_v1.5$ channel. Indeed, the U.S. Food and Drug Administration issued in 2020 a new warning regarding lamotrigine's potential to cause cardiac conduction and rhythm abnormalities because in vitro, lamotrigine behaved similar to class 1B antiarrhythmic drugs. In 2022 lamotrigine's manufacturer reassessed the data and concluded that the $Na_v1.5$ inhibition by lamotrigine was weak with rapid kinetics, not translating into clinically relevant adverse cardiac effects at therapeutic doses.[8] More recently, 2 retrospective studies that included vulnerable populations did not identify among patients with epilepsy any lamotrigine-associated deaths due to cardiac rhythm or conduction causes.[8,9] One of these studies found an apparent increase in cardiac death in a subpopulation of patients with a psychiatric indication that was attributed to overdosage and drug–drug interactions with antipsychotic agents.[8] The adventures of lamotrigine demonstrate the need for cryo-EM studies that will hopefully identify the unique features of ASM binding pockets within the brain Na_v channels.

Excitation Selectivity

Selective blockade of $Na_v1.6$ channels (encoded by *SCN8A*) in excitatory neurons was suggested to result in a better efficacy and fewer central nervous system (and cardiac) side effects. Moreover, $Na_v1.6$ inhibition may restore the impaired excitatory:inhibitory balance in Dravet syndrome associated with loss-of-function mutations in $Na_v1.1$ channels.[5] Indeed, a selective $Na_v1.6$ channel inhibitor, NBI-921352 (XEN901; Figure 1), is being evaluated as an adjunctive therapy in patients with SCN8A developmental and epileptic encephalopathy syndrome (NCT04873869, NCT05226780) and in adults with focal-onset seizures (NCT05493293). The molecular weight of this compound is considerably larger than those of currently used ASMs, potentially affecting its ability to cross the blood–brain barrier at epileptic foci in humans.[10] High-resolution identification of the binding pocket of selective sodium $Na_v1.6$ inhibitors could lead to the development of second-generation, smaller molecular-weight blockers.

Is Tighter Always Better?

Several sodium channel-blocking ASMs (phenytoin, carbamazepine, oxcarbazepine, eslicarbazepine, cenobamate) induce hepatic metabolic enzymes to various extent. Others, such as cannabidiol, inhibit them.[11] Beyond drug–drug interactions, specifically long-term enzyme induction can lead to imbalances in endogenous substrates such as vitamin D, sex hormones and cholesterol, with increased risk of vascular and other morbidities.[1] Interestingly, studies with isoform-selective $Na_v1.7$ inhibitors suggested a link between increased membrane partitioning and xenobiotic detoxification mechanisms. These include both direct enzyme inhibition and binding to nuclear receptors that mediate enzyme induction.[12] The relationships between membrane solubility and enzyme induction are in line with the higher magnitude of induction by carbamazepine than those of the less lipophilic oxcarbazepine and eslicarbazepine, as indicated by carbamazepine's higher LogD (2.7 vs 1.7 of oxcarbazepine and eslicarbazepine).[10] Cannabidiol, the most lipophilic ASM that can binds the channels from within the membrane[6] might as well be at a good position to inhibit microsomal, membrane-bound cytochrome P450 enzymes. Obtaining more detailed information on binding sites within nuclear receptors and drug-metabolizing enzymes might lead to the discovery of newer sodium channel blockers that,

similar to lacosamide, would be involved in fewer drug–drug interactions.

In conclusion, sodium channel blockers are apparently here to stay. Fine-tuning of their binding sites within the channels can set the ground for rational design of safer, more selective ASMs, and perhaps even minimize their potential to interact with other drugs.

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Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The author has served as a consultant for Biopass and TrueMed, Israel.

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