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Recent progress in sodium channel modulators for pain

Sharan K. Bagal [a,*], Mark L. Chapman [b], Brian E. Marron [c], Rebecca Prime [d], R. Ian Storer [a], Nigel A. Swain [a]

[a] Worldwide Medicinal Chemistry, Pfizer Neusentis, The Portway Building, Granta Park, Great Abington, Cambridge CB21 6GS, UK
[b] Electrophysiology, Pfizer Neusentis, 4222 Emperor Blvd., Durham, NC, USA
[c] Chemistry, Pfizer Neusentis, 4222 Emperor Blvd., Durham, NC, USA
[d] Electrophysiology, Pfizer Neusentis, The Portway Building, Granta Park, Great Abington, Cambridge CB21 6GS, UK

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ABSTRACT

Voltage-gated sodium channels (Na_vs) are an important family of transmembrane ion channel proteins and Na_v drug discovery is an exciting field. Pharmaceutical investment in Na_vs for pain therapeutics has expanded exponentially due to genetic data such as SCN10A mutations and an improved ability to establish an effective screen sequence for example IonWorks Barracuda®, Synchropatch® and Qube®. Moreover, emerging clinical data (AZD-3161, XEN402, CNV1014802, PF-05089771, PF-04531083) combined with recent breakthroughs in Na_v structural biology pave the way for a future of fruitful prospective Na_v drug discovery.

Voltage-gated sodium channels (Na_v) are a family of transmembrane ion channel proteins. Structurally, they are members of the 6-TM ion channel family and are composed of a transmembrane α-subunit of approximately 260 kDa and several associated transmembrane β-subunits of lower molecular weight. The family comprises nine members $Na_v1.1$-$Na_v1.9$ and related Na_X. Na_vs are involved in Na^+ ion conduction across cell membranes during cell membrane depolarization. If cell membrane depolarization reaches a threshold value Na_v channels open to allow Na^+ ions to flow into cells. This movement creates action potentials and nerve impulses in electrically excitable cells, for example neurons, and affects many functions for example the peripheral and central nervous system, cardiac and skeletal muscle etc. Blockade of Na_vs has been successfully accomplished in the clinic to enable control of pathological firing patterns that occur in a diverse range of conditions such as chronic pain, epilepsy, and cardiac arrhythmias. In this review we will discuss recent advances in the field of Na_v drug discovery for the treatment of pain. In particular progress in biology, structural biology, assay technology, inhibitor development and clinical success will be discussed.

Sodium channel topology and structure: Structural studies of 6TM-topology ion channels date back to the late 1980s, when Numa et al. successfully cloned α-subunits of sodium channels.[1,2] According to sequence analysis, Na_v α-subunits are composed of approximately 2000 amino acids which assemble into four distinct domains (D1–D4), each of which consists of 6TM α-helices (S1–S6) (Fig. 1). Two helices (S5–S6) from each domain contribute towards formation of the channel pore which is responsible for Na ion conduction. The S1–S4 helices from each domain form a voltage sensor which works as a sensor of change in voltage across the cell membrane. Consequently, the pore is formed from eight helices and there are four voltage sensors surrounding the pore. Between the pore-forming helices (S5–S6) from each domain there is an extended P-loop which acts to form the Na ion selectivity filter. Other voltage-gated channels, K_v and Ca_v, share common topology and mechanism of activation with the Na_v class.[3]

Recently, structural understanding of this class of ion channels has been drastically improved due to the publication of site directed mutagenesis work and several crystal structures; a $K_V1.2/2.1$ chimera crystal structure by MacKinnon[4] and multiple Na_vAb bacterial sodium channel apo crystal structures from Catterall.[5,6] Interestingly, these structures complement each other by appearing to sample several states of the Na_vAb channel. By comparison of the structural differences these structures provide valuable insight into gating mechanisms and conformational changes which occur to open the channel pore to ion flux.[5] These studies also highlight fenestration regions within the channel pore that may constitute potential binding sites for small molecule modulators. However, to date there have been no reported co-crystal structures of any sodium channel proteins with small molecule or toxin molecules bound.

* Corresponding author. Tel.: +44 7817653523.
E-mail address: sharan.bagal@pfizer.com (S.K. Bagal).

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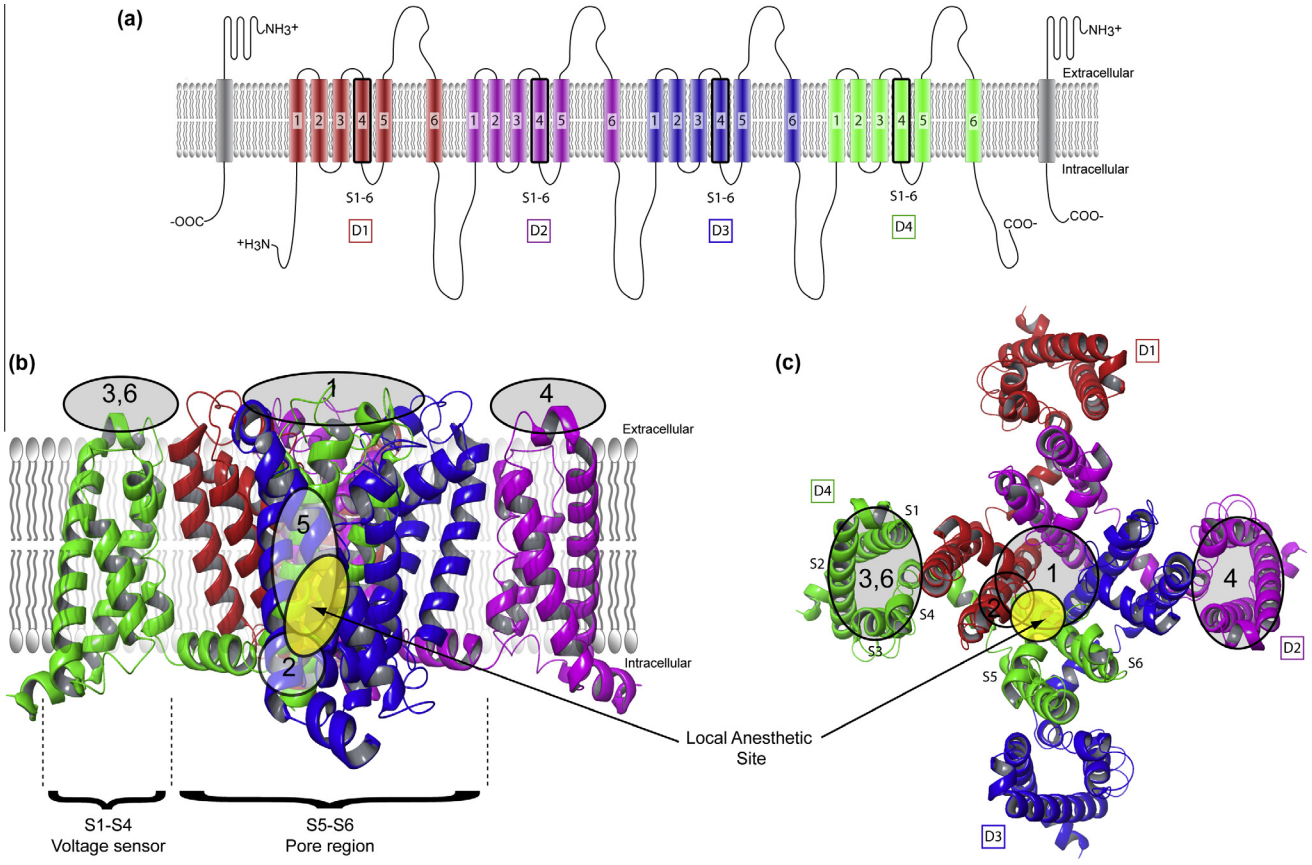

Figure 1. (a) Na_V channel structural topology. Domains D1–D4 are represented in different colors while β subunits are shown in gray. Transmembrane segments (S1–S6) labelled together with graphical representation of P-loops. (b) Side view; (c) Top view of voltage-gated sodium channel from the bacterium *Arcobacter butzleri* (Na_VAb–PDB code 3RVY)[5,6] with highlighted postulated toxin binding sites (1–6), local anesthetic binding site and significant structural features.

To further complement these recent advances in X-ray crystal structures, the field is also benefiting from advances in computational modeling and molecular dynamics to understand the opening/closing mechanism of voltage-gated channels. For example, a publication from Jensen et al., which describes micro second order molecular dynamics for the related $K_V1.2/2.1$ channels, suggests that these channels close with a hydrophobic collapse of a pore residue, which is followed by movement of the voltage-sensor domain towards the pore.[7,8] Conversely, in the opening process, the voltage sensor moves outward first, which pulls the pore domain to open.

Overall, the combination of structural, mutagenesis and computational studies have been used to build an understanding of Na_v channel conformational gating and function. At a simplistic level, Na_v channels are believed to exist in three states characterized by conduction behavior at different voltage potentials: open, closed (resting) and inactivated. At a resting *trans*-membrane potential, the channels are in a non-conducting closed state. When membrane potential is decreased (depolarization) the voltage sensors (S1–S4) move outward in a rotational movement, pulling the pore open for a short period (<1 ms). Subsequently the channel then moves via a combination of fast- or slow-inactivation processes into a non-conductive inactivated state. It is currently believed that a specific loop between D3 and D4 termed the inactivation gate is responsible for fast inactivation. Finally, an increase in membrane potential (hyperpolarization) causes the Na_v channels to return to the resting state.

Na_v channel genetics and biology: Na_v channels as pain targets gained traction with the recognition that some Na_v subtypes showed preferential or exclusive expression in peripheral sensory neurons.[9] While peripheral sensory neurons express nearly all Na_v subtypes at some level, there are three subtypes, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ which are enriched in peripheral neurons of the trigeminal and dorsal root ganglia (DRG). Of these, $Na_v1.7$ is the most broadly expressed in DRG neurons, while expression of $Na_v1.8$ and $Na_v1.9$ is largely restricted to the subset of small and medium neurons which are thought to represent the majority of nociceptors. The role of $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ in pain signaling has been well established in pharmacological, molecular and genetic studies with preclinical species.[10] Human validation of these targets had lagged behind the animal studies until the discovery that inherited erythromelalgia (IEM) is causally linked to missense mutations in $Na_v1.7$.[11] Since this discovery, the family of Na_v channelopathies has expanded in two important areas. First, the link between $Na_v1.7$ variants and clinical pain syndromes now includes a subset of idiopathic small fiber neuropathies, a disorder which affects millions of patients. Second, additional Na_v related channelopathies have been discovered which are causally linked to $Na_v1.8$ and $Na_v1.9$. These studies provide evidence for the contribution of each subtype of peripherally expressed Na_v channel to pain signaling.

$Na_v1.7$: The first Na_v channelopathy linked to pain to be identified was inherited erythromelalgia (IEM). IEM is characterized by extreme burning pain, typically in the distal extremities, which is triggered by moderate increases in temperature. Electrophysiological characterization of the mutant channels revealed a gain-of-function phenotype in channel activation, consistent with the pathological sensitivity to heating. To date, twenty SCN9A variants have been linked to IEM and have consistently shown a gain of function phenotype and demonstrated the ability to produce