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Discovery of novel sodium channel inhibitors—A gene family-based approach ☆

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Abstract

Voltage-gated sodium (Na_V) channel inhibitors are an important class of drugs that are used to treat a number of CNS indications including pain, local anaesthesia, epilepsy and bipolar disorder. These drugs all have their origins in traditional "empirical" pharmacology, and it was only some time after discovery that they were found to inhibit Na_V channels. The basis for therapeutic selectivity of these drugs within different disease indications is currently unknown. However, the subsequent discovery of a multi-gene family of Na_V channels suggests a possible mechanism and has opened the way for more targeted approaches to finding improved therapeutic inhibitors. This article describes some ongoing approaches to systematically clone, express and characterise the entire family of Na_V subtypes in order to better understand their properties and define their individual physiological and pathophysiological roles. As well as providing specific disease validation for individual subtypes, this also provides a panel of reagents for comprehensively exploring the efficacy, selectivity and potency relationships of existing Na_V-blocking drugs. In this way, a gene family-based approach to Na_V channels has enabled a "drug-to-target" approach, reversing the more usual "gene-to-target-to-drug" paradigm. Together with recent advances in assay technology, gene family-based approaches are increasing the tractability of these targets and are re-invigorating Na_V drug discovery within the pharmaceutical industry.

Keywords: Sodium channel; Gene family; Subtype profiling; Inhibitors; Drug discovery; Splice variants; Ribonuclease protection assay

1. Introduction to voltage-gated sodium channels

Voltage-gated sodium (Na_V) channels play a fundamental role in regulating the excitability of most electrically active cells. They mediate the rising phase of action potentials and have a major influence in determining the resting potential of these cells. Their indispensable role is clearly illustrated by the lethal effects of highly potent and selective neurotoxins such as tetrodotoxin (TTX), the poisonous component of pufferfish. The human genome encodes a family of 10 different Na_V subtypes that share a high degree of sequence similarity and that are highly conserved across mammalian species (Fig. 1). Sensitivity to TTX has often been used to classify Na_V channels into two major groups—those that are sensitive to nM concentrations of toxin (TTX-s), which include the major subtypes found in nervous tissue ($Na_V1.1$, 1.2, 1.3, 1.6 and 1.7) and muscle ($Na_V1.4$), and those that are relatively resistant (TTX-r) which include the cardiac subtype ($Na_V1.5$) and two other subtypes ($Na_V1.8$ and 1.9) that have a highly restricted localisation in the peripheral neurons of the dorsal root ganglion (DRG).

Molecular cloning of Na_Vs revealed them to be complex heteromeric proteins consisting of a large pore-forming α subunit together with one or more smaller β subunits (β1–4) which subtly modulate function and have a role in trafficking the channel to the cell surface (Catterall, 2000; Isom, 2001). The α subunit is organised into four homologous domains (I–IV) which each contain six transmembrane-spanning α-helical segments (S1–S6). Studies of cloned channels have identified key regions of the α subunit that are involved in mediating basic channel functions (see Fig. 2, reviewed by Catterall, 2000). For example, regions

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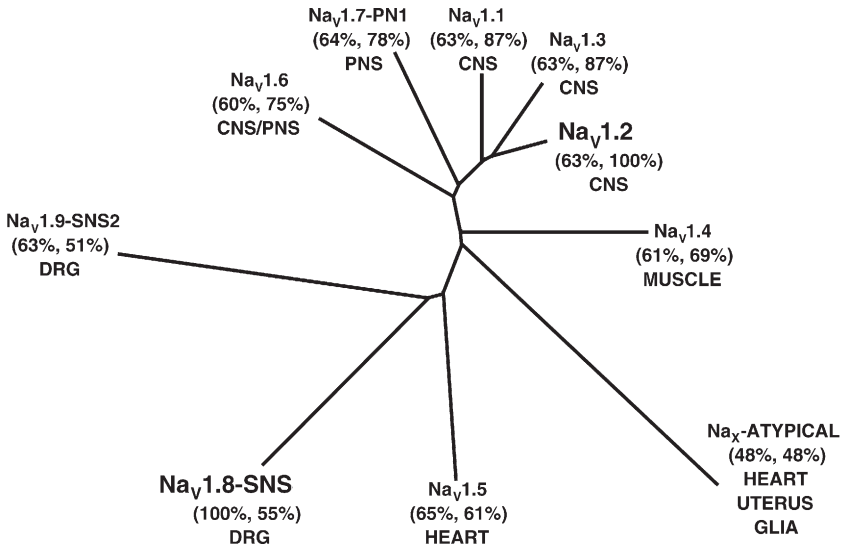

Fig. 1. Phylogenetic tree for human voltage-dependent sodium channel subtypes. The primary tissue(s) in which each subtype is expressed, and the percentage amino acid sequence identity relative to representative tetrodotoxin-sensitive ($Na_V1.2$, first value) and insensitive ($Na_V1.8$, second value) subtypes are indicated.

defining the pore (a re-entrant hydrophobic loop located between segments S5 and S6 in each domain), the voltage sensor (stretches of positively charged amino acids occurring at every third position within the helix of each S4 segment) and the inactivation gate (a cytoplasmic loop located between domains III and IV) have all been identified. Additionally, key sites of interaction with Na_V blocking drugs have been identified on the inner face of the S6 segment from 3 out of the 4 domains (Ragsdale et al., 1996; Yarov-Yarovoy et al., 2001, 2002).

1.1. Na_V channels and inherited diseases

Eight different inherited disorders have been linked to mutations in Na_V genes, including two cardiac, three skeletal muscle and three neuronal syndromes (Lehmann-Horn and Jurkat-Rott, 1999; Head and Gardiner, 2003). To date, these Na_V "channelopathies" have been found to affect four different α subunit subtypes and one β subunit subtype (Fig. 2). General features of these disorders are that they are dominantly inherited (with the exception of motor endplate disease, MED) and their phenotypes are manifested episodically. That is, the mutations remain silent until triggered by particular physiological conditions, e.g., during exercise, cold temperatures, high serum [K^+] etc. Conceivably, other potential triggers could include induction by drugs though this has not yet been demonstrated. The episodic nature of these diseases probably reflects the rather subtle effects of these mutations on channel properties. Given the fundamental role of Na_V channels in electrical signalling, this is not unexpected since mutations causing more drastic effects are likely to be lethal during development. As shown in Fig. 2, a large number of different inherited mutations have now been identified and characterised. Though they appear to be randomly scattered throughout channel, many are in or near regions involved in inactivation, including many of those associated with long QT syndrome (LQT3), potassium aggravated myotonia (PAM), paramytonia congenita (PC) and generalised epilepsy with febrile seizures plus (GEFS+). Consistent with this, functional analysis of recombinant mutant channels confirms that a number of these do indeed cause defects in inactivation and lead to an increase in persistent Na^+ currents (INaP). These can be considered as "gain-of-function" mutations, e.g., LQT3, PAM, PC, hyperkalemic periodic paralysis (HPP), GEFS+, which is consistent with their dominant mode of inheritance. In contrast, idiopathic ventricular fibrillation (IVF), severe myoclonic epilepsy of infancy (SMEI) and MED are generally due to "loss-of-function" mutations that either cause reduced currents, due to accelerated inactivation or changes in the voltage dependence of activation, or complete disruption of channel function. Interestingly, for epilepsy mutations, there appears to be a correlation between the nature of the mutation and the severity of the symptoms (Ceulemans et al., 2004). At least three different GEFS+ mutations (R1648H, T875M, W1204R) cause "gain-of-function" increases in the level of INaP suggesting a highly plausible disease mechanism.

1.2. Na_V channel blocking drugs

Despite the fundamental physiological role of Na_V channels it has been possible to develop therapeutically active Na_V inhibitors that have relatively few side effects. These Na_V blocking drugs were all discovered empirically, using traditional pharmacological methods as opposed to specifically targeting Na_Vs, and were only subsequently discovered to inhibit these channels. Given the fundamental role of Na_V channels, the question arises as to why these drugs do not cause major side effects. The availability of cloned Na_V channels has provided tools to address this question. Studies with recombinant channels suggest that three key properties contribute to the therapeutic selectivity of Na_V blockers such as lamotrigine (LTG) that, together, tip the balance towards beneficial rather than detrimental effects of inhibiting Na_V channels *in vivo* (Xie et al., 1995). Firstly, the extent of block is found to be voltage-dependent, with increased levels of inhibition occurring at more