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Using Potassium Channels As Reporters To Deconstruct The Function And Pharmacology Of Sodium Channel Voltage Sensors

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Voltage-activated sodium (Nav) channels found in both nerve and muscle cells are crucial for the generation and propagation of nerve impulses, and as such are amongst the most widely targeted ion channels by both toxins and drugs. The four voltage sensors in Nav channels have distinct amino acid sequences, raising fundamental questions about their relative contributions to the function and pharmacological sensitivities of the channel. Dissecting these contributions, however, has been problematic because the voltage sensors are contained within pseudosubunits (domain I to IV) of a single protein. Here we show that the four S3b-S4 paddle motifs within Nav channel voltage sensors can be transplanted into four-fold symmetric voltage-activated potassium (Kv) channels and can be used as reporters to individually examine the contributions of these paddle motifs to the kinetics of voltage sensor activation and their interactions with toxins. Our results show that each of the four Nav channel paddle motifs can interact with toxins from tarantula venom (PaurTx3, ProTx-I, ProTx-II, HaTx and SGTx1) or scorpion venom (AaHII and TsVII), that multiple paddle motifs are often targeted by a single toxin, and that the profiles of toxin-paddle interactions vary for different subtypes of Nav channels. The paddle motif from domain IV is unique because it slows voltage sensor activation and toxins must selectively target this motif to alter Nav channel fast inactivation. In contrast, toxins that interact with paddle motifs in domains I-III influence Nav channel opening. The influence of domain-specific interactions has important implications for developing strategies to reshape Nav channel activity. Therefore, our reporter approach and the principles that emerge will be useful in generating new drugs for treating pain and Nav channelopathies.

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Persistently "Leaky" Nav Channels In Traumatized Axons: Lowered Barriers To Nav1.6 Voltage Sensor Motions In Blebbed Plasma Membrane As A Possible Explanation

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Mechanical trauma of CNS nodes of Ranvier generates axolemmal blebs. The nodal Na^+-channel, Nav1.6, leaks Na^+ after traumatic brain injury but its molecular ''lesion'' is not understood. We found that traumatic stretch of Na^+-dye loaded HEK-Nav1.6 cells causes an immediate TTX-sensitive Na^+-leak. Also, using oocytes, we measured Nav1.6 current in cell-attached patches before and after pipette aspiration (which causes blebs) and observed irreversibly left-shifted g(V) and availability(V). To determine if intact cell Nav1.6 operation left-shifts with trauma, within-cell comparisons of HEK-Nav1.6 cell I_{Na} before and after traumatic stretch would be ideal, but this is impracticable. Instead, perforated patch recordings (multi-pulse protocols) of I_{Na} were obtained in a given HEK-Nav1.6 cell which was then swollen grossly (distilled water, 90-180 s) and returned to normal saline. After 5 min (for cell morphology and $[Na^+]_{ext}$ to regularize) I_{Na} was re-measured. The resulting pattern of I_{Na} changes at various voltages showed that, post-osmotrauma, both g(V) and availability(V) were left-shifted at least 5 mV and this was irreversible (10 min experiments). Time controls showed no left-shift. A simple explanation is that, post-trauma, abnormally fluid disorganized bilayer of blebbed membrane presents abnormally low energy barriers to Nav1.6 voltage sensor motions. Smaller depolarizations are thus required to elicit sensor repacking than in stiffer preebleb bilayer. To the extent that trauma-induced blebbing was non-uniform, left-shift would be ''smeared''. For mildly traumatized axons of the traumatic penumbra, such left-smeared Nav1.6 window current, by leaving no ''safe'' voltage, should prove even more excitotoxic than maximal left-shifting. Positive feedback in free-running axons would persistently elicit Na^+-leak as window currents from variously traumatized areas triggered each other. *Supported by CIHR and HSFO.*

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Expression, Purification and Biophysical Characterization of a Superfamily of Prokaryotic Voltage-gated Sodium Channels

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Eukaryotic voltage-gated sodium channels are monomeric membrane proteins comprised of four pseudo-repeats of domains containing six transmembrane segments, and have a molecular weight of >200 KDa. Their size and complexity makes them an arduous target for production in heterologous expression systems, a necessary step in acquiring the amounts of protein needed for biophysical and structural characterization. The simplified, single domain bacterial sodium channel containing six transmembrane segments isolated from *Bacillus halodurans*, NaChBac, can be expressed in *E. coli* in yields suitable for biophysical characterization and may enable successful crystallization and 3-D structure determination. Upon purification in 0.1% DDM, NaChBac is functional and associates to form a stable homotetramer (Nurani et al. (2008) *Biochemistry* 31:8114-8121).

Seven different bacterial sodium channels with significant homology to NaChBac have been expressed in *E. coli*, purified in high-yield and characterized for secondary structure, thermal stability and drug binding. The experimental ease of obtaining a pure and homogeneous sample varies amongst the superfamily of sodium channels studied. The bacterial sodium channels are extremely thermal stable but individual members differ in their long-term stability when stored at room temperature, 4°C and −80°C, and their ability to bind the drug mibefradil. These channels also differ in their ability to form stable tetramers upon purification in different detergents. The differences and similarities found in this superfamily of sodium channels may prove valuable for determining general structural features important for specific voltage-gated sodium channel functions. The ability to express, purify and reconstitute multiple active bacterial sodium channels in membrane-mimic environments provides an arsenal of resources for elucidating structural features and identifying residues important for sustaining function in voltage-gated sodium channels. *(Supported by a grants from the BBSRC to the MPSI consortium and BAW)*

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Chimeric bacterial-human NaV1.7 sodium channels expressed in *E. coli*

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Voltage-gated sodium channels selectively transport sodium ions across cellular membranes in response to changes in membrane potential. Prokaryotic voltage-gated sodium channels are homotetramers, each monomer containing six transmembrane helices (S1–S6), consisting of a voltage-sensing subdomain (S1–S4) and a pore-forming subdomain (S5–S6). In eukaryotes, sodium channels consist of a single polypeptide chain containing four similar domains, each with six transmembrane helices (S1–S6), which create pseudo-tetrameric channels. In humans, genetic diseases associated with the NaV1.7 sodium channel isoform include loss-of-function (i.e. channelopathy-associated indifference to pain), in addition to gain-of-function inherited painful neuropathies; hence, this channel is an important target for drug discovery.

Expression of eukaryotic membrane proteins in *E. coli* is often a difficult task, resulting in cell death, no expression of the target protein, or proteins inserted into inclusion bodies. In order to enable the expression of crucial functional regions of eukaryotic sodium channels, we have developed a method for creating chimeric proteins with the N-terminal subdomain of a prokaryotic homologue, and the C-terminal subdomain of the eukaryotic protein of interest, thereby tricking the bacterial host into expressing a protein with functional regions of interest from the eukaryote. In this study we designed, constructed, expressed, and characterised a number of sodium channel chimeras containing the voltage sensor (S1–S4) from *B. halodurans* NaChBac and the pore regions (S5–S6) from domains II and III of human NaV1.7, including the S4–S5 linkers from either the bacterial or eukaryotic protein.

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Block of Tetrodotoxin-sensitive, Nav1.7, and Tetrodotoxin-resistant, Nav1.8, Na^+ Channels by Ranolazine

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Evidence supports a role for the tetrodotoxin-sensitive Nav1.7 and the tetrodotoxin-resistant Nav1.8 in the pathogenesis of pain. Ranolazine, an anti-ischemic drug, has been shown to block cardiac (Nav1.5) late sodium current (I_{Na}). In this study, whole-cell patch-clamp techniques were used to determine the effects of ranolazine on human Nav1.7 (hNav1.7+β1 subunits) and rat Nav1.8 (rNav1.8) channels expressed in HEK293 and ND7-23 cells, respectively. Ranolazine reduced hNav1.7 and rNav1.8 I_{Na} with IC_{50} values of 10.3 and 21.5 μM (holding potential=-120 or -100 mV, respectively). The potency of I_{Na} block by ranolazine increased to 3.2 and 4.3 μM when 5-sec depolarizing prepulses to -70 (hNav1.7) and -40 (rNav1.8) mV were applied. Ranolazine (1-30 μM) caused a concentration-dependent hyperpolarizing shift in the voltage dependence of inactivation of both channels, suggesting preferential interaction of the drug with inactivated states of the channels. Ranolazine (30 μM) caused a use-dependent block (10-msec pulses at 1, 2 and 5 Hz) of hNav1.7 and rNav1.8 I_{Na} and significantly accelerated the onset of, and slowed the recovery from inactivation of both channels. An increase of depolarizing pulse duration from 3 to 200 msec did not affect the use-dependent block of I_{Na} by 100 μM ranolazine. Taken together, the data suggest that ranolazine blocks the open