

## Built for speed

### Molecular properties of the voltage sensor domain underlie the rapid activation of voltage-gated Na<sup>+</sup> channels compared with *Shaker*-type K<sup>+</sup> channels

Andrew P Braun\*

Faculty of Medicine; University of Calgary; Calgary, AB Canada

Many of us were taught in high school biology that the action potential waveform in nerves and other excitable tissues was generated by an initial rapid influx of external Na<sup>+</sup> ions across the plasma membrane, followed by an outward movement of intracellular K<sup>+</sup> ions. The former event, mediated by voltage-gated Na<sup>+</sup> channels, is responsible for the fast depolarizing upstroke of the action potential, while voltage-gated K<sup>+</sup> channels are responsible for the subsequent repolarizing phase, which largely controls action potential duration. Although Hodgkin and Huxley described the fundamental importance of this sequential activation process more than 60 y ago, the molecular and structural details underlying the faster activation of voltage-gated Na<sup>+</sup> (Nav) vs. K<sup>+</sup> (Kv) channels have yet to be fully resolved.

Insights derived from ion channel cloning and structural determination have revealed that Kv channels are comprised of 4 identical or closely related subunits, each of which contain 6 transmembrane segments (S1–6) and assemble in a symmetric, tetrameric ring complex with a centrally located ion-conductive pore. The overall structure of Nav channels follows a similar pattern, except that the four analogous subunits or domains are physically connected in series via intracellular peptide linkers to form a holo-complex. Importantly, each subunit or domain in voltage-gated K<sup>+</sup> and Na<sup>+</sup> channels contains a voltage sensor domain (VSD) comprised of segments

1–4, which undergoes activation upon membrane depolarization and drives opening of the ion conduction pathway. Whereas all 4 VSDs in Kv channels must be activated in order for pore opening to occur, Nav channel pore opening requires activation of only three VSDs, contained within domains I–III. Movement of the fourth VSD in Nav channels is delayed compared with domains I–III, and functionally, it appears to mediate fast inactivation of ionic current through the pore. Thus, part of the difference in activation speed between Nav and Kv channels may be due to the lesser number of VSDs required to move in Nav channels. However, the remaining molecular factors contributing to this difference have yet to be clearly defined. In their recent study, Bezanilla and colleagues have examined Nav and Kv channel activation using combined structure-function analysis with detailed biophysical measurements to identify critical amino acid residues that contribute to the relatively fast and slow activation rates of these 2 channel types.

Experimentally, mammalian Nav1.2 and 1.4 channels, along with Kv1.2 channels, were expressed in *Xenopus laevis* oocytes, and cut-open voltage clamp techniques were used to record ionic currents and gating currents; the latter reflect the physical rearrangements of the VSDs within each channel type. Desired replacement of key amino acids within each channel type was performed using standard site-directed mutagenesis methodology.

**Keywords:** voltage sensor domain, gating current, Nav channel, Kv channel, hydrophobicity

\*Correspondence to: Andrew P Braun;  
Email: abraun@ucalgary.ca

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Initial recordings of gating current activation in Nav1.2 and 1.4 channels vs. Kv1.2 and *Shaker*, a prototypic voltage-gated K<sup>+</sup> channel from *Drosophila*, verified that the time constants ( $\tau$  values) for Nav channel activation were 2–3-fold faster at voltages near the physiologic threshold for action potential generation (i.e., -50 to -40 mV). Moreover, co-expression of Nav channels with the widely distributed  $\beta$ 1 subunit accelerated gating current activation an additional 2-fold, leading to an overall difference of 4–6-fold between Nav and Kv channel activation. These observations support the view that the VSDs within domain I-III of Nav channels activate rapidly, whereas those in *Shaker*-type Kv channels and Nav domain IV activate more slowly.

Using primary sequence alignments of the VSDs (i.e., S1–4) from multiple Nav and Kv channels, the authors identified highly conserved amino acid differences in the S2 and S4 regions of these channels. In particular, domains I-III of fast activating Nav channel isoforms contain a key Thr residue in their S2 segments, whereas slow activating channels (i.e., Kv and voltage-gated bacterial Na<sup>+</sup> channels) contain an Ile in the equivalent position. In the S4 segments of fast Nav channels, a highly conserved Thr is present adjacent to the outermost positively charged residue, but the comparable residue is replaced by either an Ile or Val in Kv channels. Fast activating VSDs thus appear to contain hydrophilic residues in these 2 key positions, 1 in S2 and 1 in S4, whereas hydrophobic residues occupy these same positions in more slowly activating VSDs. It is noteworthy that in the domain IV VSD of Nav channels, which activates slowly, these S2 and S4 positions contain Ile and Val, respectively, and thus resemble the motifs of slowly activating Kv channels.

If these amino acid profiles truly underlie the kinetic differences observed between VSD activation in fast Nav channels vs. slower *Shaker*-type Kv and bacterial Na<sup>+</sup> channels, the authors speculated that it may be possible to create a “slow” activating Nav1.4 channel by replacing the 2 hydrophobic Thr residues in the S2 and S4 segments of domains I-III with the hydrophobic residues (i.e., Ile or Val)

identified in the S2 and S4 segments of Kv channels. A “fast” Kv channel was then constructed by replacing the 2 S2 and S4 hydrophobic residues with Thr residues.

Compared with wild-type channels, mutated “slow” Nav1.4 channels displayed slower gating current activation, with  $\tau$  values similar to those observed for wild-type *Shaker*-type Kv channels. In contrast, the time constants for gating current activation in mutated “fast” Kv channels were quantitatively similar to those in wild-type Nav1.4 channels. Based on these observations, the authors suggest that these 2 amino acid positions in the S2 and S4 segments of VSDs may represent “speed control” sites that are governed by the nature of the amino acid side-chains present at each location. Interestingly, mutated “slow” Nav1.4 channels still exhibited a -2-fold increase in the speed of gating current activation following co-expression with  $\beta$ 1 subunit, indicating that  $\beta$ 1 facilitates VSD movement independently of the “speed control” positions in the S2 and S4 segments of domains I-III.

Since replacement of hydrophobic residues normally present at the S2 and S4 “speed control” sites in *Shaker*-type Kv channels was found to accelerate VSD activation, then equivalent substitutions in the “slowly activating” domain IV of the Nav1.4 channel would be expected to have a similar effect, resulting in faster inactivation of Nav1.4 channel ionic current. Indeed, introduction of Thr residues at these 2 positions in the domain IV VSD increased the rate of ionic current inactivation up to 2-fold over the voltage range of -20 to 20 mV compared with wild-type Nav1.4 channels.

Using a variety of amino acid substitutions at the S2 and S4 “speed control” positions, the authors observed that the rates of VSD activation ( $\tau$  values) correlated negatively with the hydrophobicity of amino acid side chains present at these locations; that is, the presence of hydrophobic residues slowed down the speed of VSD activation. Interestingly, the rate of VSD de-activation also correlated negatively with the hydrophobicity of amino acids at the S2 location, but there was no kinetic influence of side-chain hydrophobicity at the S4 “speed control” site. Using Kv1.2 channel crystallographic data, the

authors suggest that hydrophobic amino acids at the S2 position raise the energy barrier for VSD movement, whereas hydrophobic amino acids at the S4 position tend to stabilize the resting state of VSDs; together, these effects would make it harder for VSDs to transition from the resting to the activated state in response to a stimulus. Hydrophilic residues at the same S2 and S4 positions have the opposite effects on these same two parameters.

Finally, the authors explored their hypothesis using a non-ion channel VSD derived from a voltage-sensitive phosphatase enzyme identified in the ocean tunicate *Ciona intestinalis* (a.k.a. sea squirt). Replacing the native Leu residue at the S4 “speed control” site in the VSD of this protein with less hydrophobic residues increased the speed of VSD-associated charge movement (i.e., gating current), without affecting the de-activation rate of this movement. These mutational data thus recapitulate those observed in Nav1.4 and *Shaker*-type Kv channels and imply that S2 and S4 “speed control” sites are a general feature of VSDs present in diverse voltage-sensitive membrane proteins. Interestingly, the authors point out that these same “speed control” sites exist within the Nav channels of a primitive, unicellular eukaryote, raising the possibility that fast Nav channel gating may have facilitated nervous system development/activity in multi-cellular organisms.

The functional contribution of S2 and S4 “speed control” sites to Nav and Kv channel activation raises the possibility that these sites may represent unappreciated targets for drugs and/or toxins that modify channel activities. Interfering with these S2 and S4 sites would be expected to impact channel gating kinetics, leading to altered action potential duration, firing frequency, and tissue excitability. Small molecules binding to these locations might thus be expected to influence membrane excitability by altering the kinetics of VSD movement. In summary, this study from the Bezanilla laboratory has provided novel and important insights into the age-old question of why Nav channels activate more rapidly compared with Kv channels. The multi-part answer appears to involve: (1) the reduced requirement for activation of 3 vs. 4 VSDs to achieve pore opening,

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(2) the kinetic influence of hydrophilic vs. hydrophobic amino acids present at the S2 and S4 “speed control” sites in each VSD and (3) an additional, independent boost to VSD activation by co-expression of Nav channel isoforms with  $\beta$ 1 subunits. Collectively, these 3 parameters appear to provide a solid structural account for a phenomenon that has puzzled biophysicists for more than half a century.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.