**Commentary**

**Sodium Channel Inactivation Goes with the Flow**

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Voltage-dependent inactivation of Na+ channels is a consequence of voltage-dependent activation (Aldrich et al., 1983), and inactivation is characterized by at least two distinguishable kinetic components: an initial rapid component (fast inactivation) and a slower component (slow inactivation). Within milliseconds of opening, Na+ channels enter a nonconducting inactivated state as the inactivation gate, the cytoplasmic loop linking domains III and IV of the α subunit, occludes the open pore (Stuhmer et al., 1989; Patton et al., 1992; West et al., 1992; McPhee et al., 1994, 1995, 1998; Kellenberger et al., 1996; Catterall, 2000). Residues that form a hydrophobic triplet (IFM) in the III-IV linker are crucial to fast inactivation (West et al., 1992), and the IFM motif has been suggested to function as a “latch” that holds the fast inactivation gate shut. Glycine and proline residues that flank the IFM motif may serve as molecular hinges to allow closure of the inactivation gate like a hinged lid (“hinged-lid model”) (West et al., 1992; Kellenberger et al., 1996). Cysteine-scanning mutagenesis of the residues I1485, F1486, and M1487 in the human cardiac Na+ channel revealed that these amino acids contribute to stabilizing the fast-inactivation particle (Deschenes et al., 1999) in analogy to the brain Na+ channel (Stuhmer et al., 1989; Sheets et al., 2000), and more recently, evidence has been presented to suggest that III-IV linker interactions with the carboxy terminal domain of the channel are also needed to stabilize the inactivated state (Motoike et al., 2004). Thus fast inactivation, in contrast with inactivation of L-type calcium channels, has been viewed as a voltage-, but not a current-, dependent process.

In contrast with fast inactivation, evidence has accumulated linking slow inactivation of Na+ channels to a current-dependent process. Slow inactivation is not affected when fast inactivation is prevented by protease treatment or when movement of the inactivation gate is blocked by specific antibodies (Vassilev et al., 1989), and therefore it is likely to be a process that is independent of fast inactivation. Moreover, transposition of all four cardiac isoform P-loops, which form (part of) the pore-lining in the selectivity filter, into the human skeletal muscle isoform (hSkM1) backbone confer heart isoform–like slow inactivation properties on the chimeric construct, suggesting a role for the P-loops in slow inactivation (Balser et al., 1996; Vilin et al., 1999).

The outer pore region of the Na+ channel protein contains highly conserved aspartate, glutamate, lysine, and alanine residues (the “DEKA” ring), which are thought to form the channel selectivity filter (Heinemann et al., 1992; Perez-Garcia et al., 1996). In analogy to ion selectivity of L-type calcium channels that contain an outer pore EEEE motif (Yang et al., 1993; Sather et al., 1994), ion selectivity in Na+ channels is likely due to a single-file ion permeation process. Mutations in the sodium-channel selectivity filter have been shown to affect gating as well as permeation and most of the effects to date have implicated slow inactivation in this process. For example, a single residue in the D-II P-loop of the cardiac Na+ channel (I891) has been shown to regulate the steady-state availability of slow inactivation (Vilin et al., 2001). Additionally, Tomaselli et al. (1995) found that mutation of a residue in the external pore mouth of the Na+ channel not only reduces single-channel conductance but also accelerates activation kinetics of the channel. There are also reports that mutations in the DEKA ring enhance the entry of Na+ channels into an ultraslow inactivated state (Hilber et al., 2001). Thus in many ways, slow inactivation of sodium channels resembles C-type inactivation of potassium channels (Liu et al., 1996), and may be coupled to ion permeation through the pore.

In this issue, Kuo et al. (2004) probe further the link between permeation and gating in Na+ channels by focusing on the kinetics of fast inactivation under conditions when the outer pore is blocked by metal ions. This work extends significantly previous studies which have indicated that both pore block by TTX and transitional metal ions such as Cd2+ and Zn2+ are coordinated by sites near the selectivity filter (Backx et al., 1992; Sheets and Hanck, 1992). Kuo et al. (2004) have systematically studied the kinetics and current dependence of block of TTX-resistant sodium channels by La3+, Zn2+, Ni2+, Co2+, and Mn2+. The blocking effect of all these multivalent ions is current dependent, and channel fast inactivation gating is particularly well correlated with this current-dependent block. Their data are particularly interesting in view of the results implicating dramatic slowing of fast inactivation with La3+.
block of the outer pore. Because the molecular components responsible for fast inactivation are located at the intracellular end of the pore, these results suggest allosteric coupling of outer pore ion binding with subtle conformational changes that must alter the inactivation process.

The results of this study thus provide further evidence that the two fundamental mechanisms of ion channels, permeation and gating, are inter-related and further, that current and voltage-dependent inactivation of channels may, in fact, be a more general process in ion channel gating than previously thought.

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REFERENCES


