It's spring-time for slow inactivation

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Some voltage-activated ion channels have evolved ways of terminating or limiting ionic flux even when a depolarized membrane potential would keep them open. When Hodgkin and Huxley observed the time-dependent decay of Na^+ permeability in the squid giant axon, they termed it "inactivation" (Hodgkin and Huxley, 1952). This process, which takes place in the millisecond timescale, typically results from the blockade of the channel pore from its cytoplasmic side by a tethered "ball" peptide encoded by the main pore-forming subunit or, in some cases, provided by an accessory modulatory subunit.

A different type of inactivation process, usually slower than the one mediated by the ball mechanism, accomplishes similar functions (i.e., stops the channel ionic conduction) typically on the timescale of seconds. The voltage-gated *Shaker* potassium channel (Papazian et al., 1987; Tempel et al., 1987) both mechanisms coexist. Hoshi et al. (1991) elegantly showed that the deletion of 46 amino acids from the N terminus (*Shaker*'s ball peptide) removes the fast inactivation process, unmasking a second inactivation process responsible for slow decay of the ionic conductance. Given its dependence on mutations in the C-terminal region, the slow inactivation is also commonly reported as C-type. Slow inactivation does not seem to involve blocking particles or ball peptides; rather, it has been associated with a rearrangement of the outer pore, likely involving the selectivity filter. The evidence from a plethora of studies and approaches suggests that slow inactivation correlates with conformational rearrangement optically resolvable from the extracellular side of transmembrane segments (Cha and Bezanilla, 1997; Loots and Isacoff, 1998). In addition, prolonged depolarizations modify the voltage dependence of the charge movement process, likely affecting the slow inactivation process (Olcese et al., 1997, 2001). See Kurata and Fedida (2006) for a review on inactivation mechanisms.

What really is slow inactivation? Although one can envisage the mechanism of fast inactivation, where a channel is inactivated by a tethered peptide docking into the conduction path, the mechanism underlying slow inactivation remains elusive. An intriguing feature of C-type inactivation, which reinforces its intimate relationship with the selectivity filter of the K^+ pore, is that permeant ions that have a longer sojourn within the selectivity filter also reduce the rate of inactivation (López-Barneo et al., 1993). Similarly, the presence of fast blockers sitting on the outer pore, such as TEA ions, significantly slows down the rate of the inactivation process (Grissmer and Cahalan, 1989; Choi et al., 1991), as if the presence of the blocker prevents a "collapse" of the pore and maintains the conducting state by keeping a "foot in the door." Indeed, TEA block and C-type inactivation have a common friend, residue 449 in *Shaker* channel. External TEA affinity is dramatically enhanced by substitutions at this position of aromatic residues (phenylalanine or tyrosine). Moreover, cysteines substituted in the same position 449 have also been found to drift toward the center of the conduction path in C-type–inactivated *Shaker* channels (Yellen et al., 1994), revealing conformational rearrangements taking place during the slow inactivation process.

The high-affinity aromatic TEA-binding site was proposed to be formed by four pore-lining aromatic groups at the 449 *Shaker* position, mediated by a charge-quadrupole interaction between the cationic pore blocker and the π electron orbitals (Heginbotham and MacKinnon, 1992). Thus, an en face orientation of the four aromatic rings toward the TEA was expected to bring about the coordination of TEA at the center of the pore. However, the elucidation of high-resolution KcsA channel structure, a prokaryotic K^+ channel homologous to *Shaker*, revealed that its four tyrosines at position 82 (corresponding to *Shaker* 449) did not really point their π orbitals toward the central axis of the pore (Doyle et al., 1998). Furthermore, when Lenaeus et al. (2005) cocrystallized KcsA with a TEA analogue, they found it within van der Waals interaction range with the edge of Tyr82 aromatic rings, a geometry inconsistent with the en face model of a cation π–orbital molecular interaction.

The authors of the paper that this commentary focuses on (see Ahern et al. in this issue) have previously challenged the tendency to overgeneralize about K^+

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pore structure by studying TEA block in the *Shaker* channel and providing convincing experimental and theoretical evidence that, in the *Shaker* channel, a TEA molecule does experience a cation–π interaction with four Phe residues at position 449 arranged in the en face configuration (Ahern et al., 2006). The implications of their results go beyond the mechanisms for external TEA block and raise issues about the variability and flexibility of pore structures, as well as the treatment and merging of structural and electrophysiological data from different pores. Indeed, they probably uncovered the tip of an iceberg (Roux, 2006). And they have done it again: their study (Ahern et al., 2009) elegantly addresses the relation between pore structure and slow inactivation in the *Shaker* channel, while keeping TEA in the toolbox.

The experimental approach uses the in vivo nonsense suppression technology (Nowak et al., 1998) to incorporate progressively fluorinated Phe analogues at position 449 of a *Shaker* channel lacking fast inactivation. The fluorines withdraw π electrons from the aromatic ring, reducing the negative electrostatic potential on its face. This strategy offers a superb control to manipulate the strength of the cation–π interaction.

Ahern et al. (2009) find that TEA accelerates slow inactivation in the *Shaker* mutant T449F, an effect opposite to the canonical foot-in-the-door effect, as if the putative inactivation gate were pulled closed by the presence of the TEA, a process that the authors called "spring-in-the-door." The serial fluorination of Phe 449 accelerated the rate of channel inactivation and progressively restored the TEA foot-in-the-door effect, strongly suggesting that the spring-in-the-door effect is mediated by the cation–π interactions. The results are fascinating and consistent with the view that the four Phe 449's orient the negatively charged faces of their aromatic side chains toward the center of the pore.

In the absence of charged TEA, the four Phe in en face configuration would experience an electrostatic repulsion opposing their motion toward the central axis of the pore, thus slowing the progression of inactivation. On the other hand, the electrostatic attraction of TEA⁺ with the π orbitals can explain the acceleration of the inactivation rate (the spring-in-the-door). This is certainly a plausible interpretation of the finding, which the authors investigate further.

The TEA spring-in-the-door effect on the T449F mutant disappears by adding a second mutation (V438A), which in fact restores the foot-in-the-door effect in V438A/T449F channels and reduces TEA affinity. The authors explain that V438A may reorient F449 from en face to edge-on. Is this possible? Intriguingly, the *Shaker* channel's prokaryotic relative KcsA (Doyle et al., 1998) can undergo time-dependent reduction of conductance with all the symptoms of C-type inactivation (Cordero-Morales et al., 2007). In one of the crystal structures of a

KcsA mutant, in which inactivation is impaired (E71A, homologous to *Shaker* 438), the side chain of Y82 (449 in *Shaker*) shows an en face orientation (Cordero-Morales et al., 2006), unlike what is seen in wild-type structures. Although this scenario is opposite to the one proposed for V438A in S*haker*, it underlines the importance of this position in the regulation of pore stability (Cordero-Morales et al., 2007) but, most importantly, supports the notion that the K⁺ pore is a highly dynamic structure allowing for subtle conformational transitions with macroscopic effects on conduction. The interpretation of the experimental findings is substantiated by ab initio quantum mechanical calculations that are consistent with the idea of the reorientation of four Phe 449's.

One of the merits of this work is to have proposed a comprehensive mechanism for apparently different aspects of the K⁺ pore properties, offering a reasonable mechanistic view of pore block, inactivation process, and structural rearrangement, and inferring an unexpected plasticity of the K⁺ conduction pathway. The proposed convergence of Phe 449's toward the center of the pore, as proposed in the quantum mechanical calculations, is in line with the idea of the collapse of the pore during slow inactivation. But what does a slow-inactivated channel look like? Currently, there are no solved atomic structures of an open-inactivated channel. Nonconductive or "pinched" structures at the level of the selectivity filter have been proposed (Lenaeus et al., 2005; Cordero-Morales et al., 2007), which may provide working models to understand slow inactivation.

The work of Ahern and colleagues necessarily has limits and does not purport to explain the totality of pore mutation effects in *Shaker* and other K⁺ channels. Nevertheless, one can hardly imagine a less invasive way to experimentally test the proposed hypotheses. The work certainly has brought to the fore intriguing aspects of the potassium pore dynamics, and we eagerly await subsequent research that will build on this foundation.

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