PERSPECTIVES

Resurgent currents turn painfully exciting

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The discovery of resurgent currents in 1997 (Raman & Bean, 1997) added a new dimension to one of the most thoroughly investigated gating processes of voltage-gated sodium channels: the fast inactivation. In response to a depolarizing change in membrane potential, sodium channels activate quickly, which leads to the initiation of an action potential in excitable cells. Within milliseconds of opening, the channel pore is occluded by inactivation via the DIII–DIV linker, allowing repolarization to occur. During this fast inactivation process, the intracellular inactivation linker containing the IFMT motive docks on its receptor sites at the cytoplasmic face of the channel pore. With the linker bound, the channel is no longer permeable to sodium ions and hyperpolarized potentials are required in order to open the channel again.

In some cell types this process of fast inactivation competes with an intracellular particle, probably the c-terminal portion of the $\beta 4$ subunit of sodium channels, that binds to the open channel pore. Upon repolarization, this blocking particle dissociates from the channel, allowing a resurgent current to flow and depolarize the membrane, favouring repetitive neuronal firing.

Until recently resurgent currents were only detectable in native neurons such as Purkinje neurons or dorsal root ganglion cells (Raman & Bean, 1997; Cummins *et al.* 2005), and Nav1.6 was thought to play the major role in its generation. Last year Jarecki *et al.* (2010) demonstrated that naturally occurring mutations that slow the rate of inactivation decay could also induce resurgent currents in other channels. Resurgent currents were dramatically enhanced by mutations in Nav1.7 associated with the chronic pain syndrome paroxysmal extreme pain disease (PEPD) in humans, suggesting a role of resurgent currents in the pathophysiology of pain.

Unfortunately resurgent currents are not normally detectable in transfected cell lines and therefore not easily accessible to molecular examination. Resurgent currents had only been recorded from the heterologously expressed sodium channels Nav1.5 (Wang *et al.* 2006) and Nav1.1 (Aman *et al.* 2009). In an article in a recent issue of *The Journal of Physiology*, Theile *et al.* (2011) have managed for the first time to record resurgent currents from HEK cells transfected with Nav1.7, when the c-terminal portion of $\beta 4$ is added to the pipette solution (as was necessary for Nav1.1 and 1.5). They examined naturally occurring mutations of Nav1.7 linked to two neuropathic pain syndromes: PEPD and erythromelalgia. Interestingly, the PEPD mutations displayed larger resurgent currents than wild-type (WT), but this was not the case for mutations linked to erythromelalgia, underscoring the importance of the site of mutation for the molecular mechanisms of resurgent current generation. Erythromelalgia mutations speed up activation whereas PEPD mutations slow down the inactivation process. It seems intuitive that PEPD mutations would favour the open channel block by the proposed blocking particle, supporting the generation of resurgent currents. Indeed, the authors describe a strong correlation between the inactivation decay time constant and the size of the resurgent currents mediated by Nav1.7. The more sluggish fast inactivation is, the higher is the likelihood for this channel to produce resurgent currents in the presence of the $\beta 4$ peptide. Due to its fast blocking of the open channel, $\beta 4$ peptide speeds up inactivation time constants, and the amount of this ultra-fast open channel block by the $\beta 4$ peptide turned out to be a good predictor of the relative rate of normal fast inactivation.

PEPD mutations occur within the inactivation gate itself, but also on the IFMT docking sites of Nav1.7. Resurgent currents are larger when the inactivation gate is impaired compared to when the docking sites are affected. Theile *et al.* propose that if one docking site is mutated, IFMT might still be able to bind to an alternative docking site thereby out-competing $\beta 4$, allowing inactivation to still occur, albeit somewhat slower. When IFMT itself is affected, its biding to any docking site is impaired, increasing the likelihood of $\beta 4$ binding. More mutagenesis experiments are needed to strengthen this hypothesis of the molecular mechanism of the ultra-fast open channel block and to potentially identify the single amino acids responsible for its occurrence. On the back of the description of resurgent currents mediated by Nav1.7 in a heterologous expression system by Theile *et al.* such experiments are likely to be feasible.

It is still puzzling that cotransfection of $\beta 4$ itself does not suffice to induce resurgent currents mediated by Nav1.7 in HEK293 cells. It is possible that in native neurons the $\beta 4$ subunit is cleaved by internal enzymes (as has been suggested for example by Huth *et al.* 2011) and that these might otherwise be lacking or reduced in number in cell lines. Alternatively, it may be that in the native environment further modification of the peptide occurs.

Although the phenotypes associated with erythromelalgia and PEPD are both attributable to gain-of-function mutations in Nav1.7, they differ substantially in their clinical picture. This discrepancy might be explained by the molecular mechanism of facilitating activation on the one hand and favouring resurgent currents on the other. More studies are needed to establish how a specific type of molecular gain of function might account for particular clinical features such as type of trigger events (warmth for erythromelalgia, cold or mechanical stimuli for PEPD), distribution of symptoms across the body, age of onset and the type of pain. Answers to these questions will help to develop more specific pain killers with well described molecular targets.

References

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