PERSPECTIVES

$Na_v1.9$, G-proteins, and nociceptors

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It is well known that inflammatory mediators, when introduced into peripheral tissues, can trigger pain. This appears to be due, at least in part, to depolarization and increased excitability of nociceptive dorsal root ganglion (DRG) neurons, which innervate peripheral tissues such as the skin (England *et al.* 1996). Precisely how these changes occur is not yet fully understood. DRG neurons express multiple isoforms of sodium channels, including sodium channel $Na_v1.9$ (Dib-Hajj *et al.* 1998), which is preferentially expressed in nociceptors (Fang *et al.* 2002). $Na_v1.9$ exhibits unique electrophysiological and pharmacological properties, including resistance to 250 nM tetrodotoxin (TTX), substantial overlap of activation and steady-state inactivation which bracket resting potential, and very slow activation and inactivation kinetics that enable it to produce a persistent sodium current (Cummins *et al.* 1999). As a result of these physiological properties, $Na_v1.9$ is not a major contributor to the rapid depolarizing phase of the action potential, but rather contributes to setting the electrogenic properties of nociceptor DRG neurons by modulating their resting potentials and amplifying their responses to subthreshold stimuli (Herzog *et al.* 2001).

Inflammatory mediators, acting via a G-protein-dependent mechanism most likely involving $G_{i/o}$, increase the $Na_v1.9$ sodium current (Rush & Waxman, 2004). Baker *et al.* (2003) previously showed that G-protein-triggered up-regulation of the persistent $Na_v1.9$ current can produce changes in membrane excitability sufficient to cause spontaneous activity, even at a membrane potential near –60 mV. Now, in the current issue of *The Journal of Physiology*, Östman *et al.* (2008) provide the important observation that DRG neurons from $Na_v1.9$ knock-out mice, in which the TTX-resistant persistent $Na_v1.9$ current is absent, do not display up-regulation of persistent Na^+ current or an increase in excitability when exposed to GTPγS. Expression of a human clone of $Na_v1.9$ within DRG neurons restored these properties. These observations add to the evidence implicating $Na_v1.9$ as a critical molecule in the response of DRG neurons to inflammatory mediators.

The depolarizing effect of $Na_v1.9$ on resting potential (Herzog *et al.* 2001) and the up-regulation of $Na_v1.9$ by GTP (Baker *et al.* 2003) predict that GTP should modulate resting potential of nociceptive DRG neurons, and this was observed by Baker *et al.* (2003). This implies that resting potential in these cells is not fixed but, on the contrary, is likely to be state-dependent. The changes in resting potential would, in turn, be expected to flip nociceptive DRG neurons and their axon terminals into more, or less, excitable states, and could thus provide a powerful mechanism for sensitization or awakening of previously silent nociceptors. The changes in resting potential could be differentially distributed in different neuronal compartments (cell body, axon shafts, axon terminals) even in the face of a uniform pattern of expression of $Na_v1.9$ (if that is the case), because of regional differences in the concentration of GTP and/or G-proteins.

An unexplained finding by Östman *et al.* (2008) and in the earlier results of Baker *et al.* (2003) is that GTP-induced up-regulation of $Na_v1.9$ is associated with a significant reduction in voltage threshold (defined as the value of potential where there was a clear deviation from a passive response, leading to an all-or-none action potential) in addition to a reduction of current threshold (the amount of injected current needed to evoke an action potential). Since the sodium channel isoforms involved in the rapid upstroke of the action potential do not include $Na_v1.9$ (due to its slow activation kinetics; Herzog *et al.* 2001), it is not immediately clear how GTP-induced up-regulation of $Na_v1.9$ would lower the voltage threshold. In a somewhat analogous situation provided by the hereditary painful disorder erythromelalgia, gain-of-function mutations of $Na_v1.7$, another sodium channel that operates in the subthreshold domain, effectively increased the current produced by this sodium channel isoform. In DRG neurons expressing these $Na_v1.7$ mutations, current threshold is reduced, but voltage threshold is unchanged compared to neurons expressing wild-type channels (Dib-Hajj *et al.* 2005; Rush *et al.* 2006). This result would be expected for an increase in current from a channel that largely operates in the subthreshold range.

One possible explanation for the drop in both voltage threshold and current threshold after GTP-induced up-regulation of $Na_v1.9$ is that GTP might modulate multiple channels within DRG neurons. If, for example, GTP up-regulated, or unmasked, low-threshold rapidly activating sodium channels that contribute to the action potential upstroke, both voltage threshold and current threshold could be reduced. Moreover, as a result of its slow kinetics, $Na_v1.9$ produces a response that can outlast depolarizing stimuli. The prolonged response of $Na_v1.9$ could maintain a depolarizing drive long enough to inactivate low-threshold potassium channels and tip the balance of inward to outward currents needed to evoke a regenerative response at lower potentials.

The expression of TTX-resistant persistent currents, at the nanoamp levels observed by Östman *et al.* (2008) after intranuclear injection or electroporation of a human $Na_v1.9$ clone in $Na_v1.9$ knockout DRG neurons, is notable, especially because, like other workers using other $Na_v1.9$ constructs, they did not observe robust TTX-resistant currents in transfected heterologous cells such as HEK293 cells using the same vector. While not identifying the auxiliary subunits or modulatory factors that support expression of functional $Na_v1.9$ channels in DRG neurons, these results hint at the presence, within a subset of DRG neurons, of such cell-specific factors. Contactin has been shown to bind to $Na_v1.9$ and participates, possibly by linking the channels to tenascin-C, in the surface localization of $Na_v1.9$ channels in DRG neurons and their non-myelinated axons, which include nociceptors (Liu *et al.* 2001) and an even more complex combinatorial arrangement involving additional molecules, potentially modulated by G-proteins, may direct the specific pattern of expression of $Na_V1.9$ in nociceptors, as annexin II/p11 does for

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$Na_v1.8$ (Okuse *et al.* 2002). The results of Östman *et al.* (2008) add to the evidence suggesting that the molecules that constitute this $Na_v1.9$-permissive cell background may be identifiable within the DRG neuron transcriptome.

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