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Na⁺ channel blockers for the treatment of pain: context is everything, almost

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Voltage-gated Na⁺ channels (VGSCs) remain viable targets for the development of novel analgesics for two main reasons. First, because of they underlie the upstroke of the action potential, a block of these channels results in a block of neural activity and therefore a block of pain. Second, functional diversity and selective expression patterns of channel types indicate that it may be possible to selectively block persistent pain associated with peripheral tissue injury. Importantly, this block may be possible with little impact on acute pain or low threshold mechanosensation, and no cardiac or central nervous system side effects.

A single alpha subunit is necessary and sufficient to generate a functional VGSC. The VGSC alpha subunit is a large molecule (>200 kd) encompassing 4 domains (I–IV) with similar structures that consist of 6 transmembrane segments (S1–S6) (Catterall, et al., 2005). The fourth segment in each domain contains the voltage sensor. A loop between S5 and S6 lines the channel pore and confers ion selectivity. A unique hydrophobic sequence, the IMF motif, in the intracellular loop between domains III and IV has access to and can block the inner mouth of the pore when the channel is open. The IMF mediated block of open channels mediates channel inactivation. Nine alpha subunits (NaV1.1 – NaV1.9) have been characterized. A tenth alpha subunit (Na_X) has been identified, but it does not appear to function as a voltage-gated channel (Catterall, et al., 2005).

Unique features of each of the alpha subunits are largely responsible for unique biophysical properties of the channel (i.e., the voltage range over which the channel begins to open, the voltage-dependence and rate of inactivation, the rate of recovery from inactivation) and therefore channel function. However, the alpha subunits do associate with beta subunits, 4 of which have been identified, that may also influence the channel properties, density and distribution within a cell (Isom, 2000, Yu, et al., 2003). Several other molecules have been identified that appear to play an important role in VGSC trafficking to the cell membrane including Annexin II/p11, contactin, and clathrin-associated protein-1A (CAP-1A). Annexin II/p11 is involved in trafficking Na_V1.8 channels to the cell membrane (Okuse, et al., 2002). Contactin, associates directly with, and regulates the density of, Na_V1.3 (Shah, et al., 2004) and Na_V1.9 (Liu, et al., 2001); although data from a contactin null mutant mouse suggests the role of this protein may be more complicated as the knockout resulted in a reduction in both Na_V1.8 and Na_V1.9 in primary afferent cell body and axons, but no change in TTX-sensitive current or channels (Rush, et al., 2005). CAP-1A serves to link Na_V1.8 to clathrin and appears

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to contribute to the clathrin mediated removal of this channel from the cell membrane (Liu, et al., 2005).

The density and distribution of channels both within and between tissues may be a particularly important determinant of channel function (Lai, et al., 2004). For example, Nav1.4 and Nav1.5 are largely restricted, in the adult, to skeletal and cardiac myocytes and are therefore, responsible for the excitability of these tissues. Similarly, Nav1.1 and Nav1.3 are primarily localized to the soma of neurons in the central nervous system where they function to integrate synaptic activity, while Nav1.6 is localized in nodes of Ranvier in myelinated axons in both the central and peripheral nervous system where it underlies action potential propagation. Finally, alpha subunits also contain a number of phosphorylation sites, particularly in the intracellular linker between domains I and II that enable dynamic regulation of channel properties (Chen, et al., 2006): depending on the subunit, channels have been shown to be up or down regulated by second messenger pathways involving the activation of protein kinase A (Cantrell and Catterall, 2001, Fitzgerald, et al., 1999, Gold, et al., 1998), protein kinase C (Cantrell and Catterall, 2001, Gold, et al., 1998), sphingomyelinase (Zhang, et al., 2002), calmodulin (Herzog, et al., 2003), and p38 MAP kinase (Jin and Gereau, 2006, Wittmack, et al., 2005).

If the selective distribution and essential role in action potential generation were not enough motivation for the development of selective VGSC blockers for the treatment of pain, there is evidence that several VGSC alpha subunits may be responsible for pain associated with tissue injury. The first channel to be implicated was Nav1.3 which is normally only present in the peripheral nervous system during embryogenesis. However, there is a dramatic increase in the expression of this alpha subunit following peripheral nerve injury (Waxman, et al., 1994). A unique feature of this channel is that it recovers relatively rapidly from inactivation (Cummins, et al., 2001). Thus, the increase in expression combined with unique biophysical properties led to the natural hypothesis that this channel contributes to paraesthesias, dysaesthesias and ongoing pain that are hallmarks of peripheral neuropathy. Consistent with this hypothesis, there is evidence that selectively knocking down expression of this channel with antisense oligodeoxynucleotides attenuates peripheral nerve injury-induced mechanical hypersensitivity (Hains, et al., 2004). More recent data (Lindia, et al., 2005, Nassar, et al., 2006), however, has led investigators to question the role of this channel in neuropathic pain.

A second VGSC directly linked to pain associated with tissue injury is Nav1.7. Compelling evidence in support of a causal link between this channel and pain comes from the discovery of individuals harboring both gain-of-function and loss-of-function mutations in the channel. Two different gain-of-function mutations are associated with two distinct pain syndromes. One of these, erythromalgia is a rare disorder associated with pain and redness in the feet and hands (Yang, et al., 2004). Several different Nav1.7 mutations have been identified in patients with erythromalgia, all of which appear to be associated with an increase in channel function (Waxman and Dib-Hajj, 2005). Interestingly, at least one of the mutations results in a depolarization of resting membrane potential which both increases the excitability of primary afferent neurons and decreases the excitability of sympathetic postganglionic neurons (Rush, et al., 2006); it is the combination of these two changes that is likely to account for the striking symptoms of this disorder. The second gain-of-function mutation in Nav1.7 is associated with paroxysmal pain disorder (Fertleman, et al., 2006). This mutation results in a decrease in the rate and/or extent of channel inactivation. The result is a Na⁺ current that persists following repolarization of the action potential which serves as the driving force for subsequent action potential generation. As with erythromalgia, paroxysmal pain disorder impacts specific body regions, most commonly the anal sphincter, despite the fact that the mutant channels are expressed throughout the peripheral nervous system. This observation serves as a reminder that despite their importance in controlling neuronal excitability, VGSC do not function in

isolation as other mechanisms appear able to mitigate the impact of the channelopathy in unaffected tissue. That said, the loss of function mutation of Nav1.7 results in what appears to be complete insensitivity to pain (Cox, et al., 2006). Interestingly, individuals harboring this mutation exhibit few, if any, detectable somatosensory abnormalities.

Analysis of the cohort with a loss of function mutation in Nav1.7 supports a role for this channel in acute nociceptive processing and several additional lines of evidence support a role for this channel in inflammatory hyperalgesia: Nav1.7 mRNA (Black, et al., 2004) and protein (Black, et al., 2004, Gould, et al., 2004) are increased in the presence of inflammation in association with an increase in TTX-sensitive current (Black, et al., 2004); inflammatory hyperalgesia is attenuated following knock-down of the channel in rats (Yeomans, et al., 2005); and inflammatory hypersensitivity is either absent or dramatically attenuated in mice in which the channel was selectively knocked out in nociceptive afferents (Nassar, et al., 2004). Evidence against a role for Nav1.7 in neuropathic pain was suggested by the observations that in patients with peripheral nerve injury Nav1.7 protein appears to decrease in the DRG neurons and remains undetectable in injured peripheral nerve (Coward, et al., 2001) and that there appears to be no deficit in the nerve injury-induced hypersensitivity in nociceptors specific Nav1.7 null mutant mice (Nassar, et al., 2004). More recently, however, an Nav1.7 selective blocker was described (Hoyt, et al., 2007) that is said to reverse hypersensitivity in animal models of nerve injury (Hoyt, et al., 2007).

A third VGSC that appears to play a critical role in pain associated with tissue injury is Nav1.8, the focus of the study by Yamane and colleagues in this issue (Yamane, et al., 2007). What makes Nav1.8 a particularly attractive target for the development of a selective channel blocker is that it is only expressed in sensory neurons (Akopian, et al., 1996, Sangameswaran, et al., 1996) and among these, it is largely restricted to nociceptive neurons (Djouhri, et al., 2003). Furthermore, within nociceptive neurons, it appears to be primarily targeted to terminals and the cell body, suggesting a role in action potential initiation (Lai, et al., 2004). The biophysical properties of the channel are also consistent with a role in mediating sustained activity in the presence of persistent membrane depolarization as would be expected in the presence of inflammatory mediators (Elliott and Elliott, 1993). In fact, the presence of this channel is thought to be the critical link enabling the hyperexcitability of nociceptive afferents observed in the presence of Nav1.7 mutations associated with erythralgia (Rush, et al., 2006).

As part of the rationale for focusing their study on Nav1.8, Yamane and colleagues summarize data implicating the involvement of this channel in the hyper-reflexia and hypersensitivity observed following inflammation of visceral structures. For example acetic acid-induced bladder hyper-reflexia is attenuated following antisense knock-down of Nav1.8 (Yoshimura, et al., 2001). Additional evidence that Nav1.8 mediated currents play an important role in visceral hyperalgesia and hyper-reflexia comes from the observations that these currents are increased in sensory neurons 1) innervating the stomach following gastric inflammation (Bielefeldt, et al., 2002), 2) innervating the jejunum/abdominal cavity, following upper gastrointestinal tract infection *Nippostrongylus brasiliensis* (Hillsley, et al., 2006), and 3) innervating the ileum (Stewart, et al., 2003) and 4) colon (Beyak, et al., 2004) following trinitrobenzene sulphonic acid (TNBS) induced inflammation of these structures. Nav1.8 mediated currents in colonic sensory neurons are increased by inflammatory mediators (Gold, et al., 2002). Furthermore, neuronal hyperexcitability following infection of the jejunum is attenuated in Nav1.8 null mutant mice (Hillsley, et al., 2006) as is the hyperalgesia and nociceptive behavior associated with intracolonic capsaicin (Laird, et al., 2002).

There is also a growing body of evidence in support of a role for Nav1.8 in the hyperexcitability and pain associated with injury to other somatic structures. Nav1.8 current is increased in

somatic sensory neurons by a host of proinflammatory mediators including adenosine (Gold, et al., 1996), endothelin (Zhou, et al., 2002), nerve growth factor (Zhang, et al., 2002), prostaglandin E₂ (Gold, et al., 1996), serotonin (Cardenas, et al., 1997, Gold, et al., 1996), and TNF- α (Jin and Gereau, 2006) utilizing second messenger pathways ranging from PKA (Gold, et al., 1998) to p38 MAPK (Jin and Gereau, 2006). There is even an increase in channel density in painful tooth pulp (Renton, et al., 2005, Warren, et al., In Press). Antisense knock down of Nav1.8 attenuates both the development (Khasar, et al., 1998) and maintenance (Joshi, et al., 2006, Porreca, et al., 1999) of inflammatory hyperalgesia. Importantly, there is also compelling evidence to suggest that the Nav1.8 is critical for the manifestation of neuropathic pain. The channel is sensitized in association with diabetic neuropathy (Hirade, et al., 1999) and accumulates in painful neuromas following traumatic nerve injury (Coward, et al., 2001, Coward, et al., 2000). Interestingly, the manner in which this channel appears to contribute to neuropathic pain changes over time with a role in the uninjured neighbors of injured afferents at early time points (Gold, et al., 2003) and injured afferents at later time points (Coward, et al., 2000). While not tested at later time points, antisense knock-down of the channel can both prevent and reverse hypersensitivity observed following traumatic nerve injury (Gold, et al., 2003). It is important to point out, particularly within the context of the study conducted by Yamane and colleagues, that decreasing Nav1.8 may effectively attenuate visceral hyper-reflexia, even if this channel is not the primary mechanism mediating the increase in excitability. For example, bladder inflammation results in a dramatic increase in the excitability of bladder afferents that appears to reflect a decrease in voltage-gated K⁺ channels (Yoshimura and de Groat, 1999), yet knocking down Nav1.8 effectively reverses the inflammation-induced hyper-reflexia (Yoshimura, et al., 2001).

While it is clear from the preceding discussion that a selective blocker of VGSCs such as Nav1.7 or Nav1.8 may have tremendous therapeutic potential, such selectivity has been difficult to come by. One major reason for the difficulty in identifying α -subunit specific blockers is that there is considerable homology between α subunits at sites most logically targeted. For example, the channel pore is effectively and selectively blocked with macromolecules such as tetrodotoxin (TTX) and saxitoxin, yet only a single amino acid distinguishes toxin sensitive (Nav1.1–1.7) from resistant (Nav1.8–1.9) channels in this region (Sivilotti, et al., 1997). Similarly, immobilizing the voltage-sensor can prevent channels from opening, yet compounds such as local anesthetics that utilize such a mechanism are relatively non-selective for channels in the resting or closed state (Gold, et al., 1998). That said, detailed analysis of the mechanisms of local anesthetic actions led to the concept of a state-dependent block, whereby the potency of these compounds depends on the state of the channel (Hille, 1977): these compounds have access to a high affinity site when channels are in an open or inactivated state, while only a low affinity site is accessible to these compounds when channels are in a resting or closed state. Taking advantage of this property, even non-selective compounds such as lidocaine may be used systemically to block channels demonstrating aberrant activity, such as those in the heart responsible for arrhythmias, or those in sensory nerves underlying ongoing pain.

Ralfinamide (NW-1029), the compound employed in the study by Yamane and colleagues, was identified in a screen of potential Na⁺ channel blockers (Veneroni, et al., 2003). Like local anesthetics, ralfinamide-induced block of VGSCs is state dependent (Stummann, et al., 2005). The potency for block of channels in the inactivated state is up to 7 times higher than channels in a resting state. There is little difference between TTX-sensitive currents and Nav1.8 mediated currents with respect to the potency of ralfinamide for channels in resting or inactivated states. However, this compound appears to selectively facilitate use-dependent block of Nav1.8 channels (Stummann, et al., 2005). This phenomenon appears to reflect a more rapid entry into and a slower recovery from an inactivated state, with more channels residing in an inactivated state with each successive activation of the channels. And it is this difference

in the fraction of channels inactivated with each activation that appears to confer the selectivity of ralfinamide. Importantly, ralfinamide was shown to reverse hypersensitivity in animal models of neuropathic and inflammatory pain (Veneroni, et al., 2003).

To further explore the contribution of $\text{Na}_V1.8$ to the antinociceptive effects of ralfinamide, Yamane and colleagues characterized the impact of this compound on the excitability of isolated DRG neurons in the presence of TTX. Excitability was assessed with current injection and the number of evoked action potentials was used to monitor the effects of ralfinamide. Subpopulations of neurons were defined by the number of action potentials evoked in response to suprathreshold stimuli as tonic (>4 action potential over 600 ms) or phasic (< 4 action potentials over 600 ms). Subpopulations were also defined by responsiveness to the algogenic compound, capsaicin (500 nM).

Three intriguing observations arose from their study. First, ralfinamide preferentially reduced the number of evoked spikes in tonic capsaicin responsive neurons, having a significantly smaller effect on tonic capsaicin unresponsive neurons. Second, substance P selectively increased the number of evoked action potentials in capsaicin responsive neurons. And third, repetitive spiking induced by substance P in capsaicin responsive neurons was selectively attenuated by ralfinamide. These observations are intriguing for at least two reasons. First, they indicate that the properties of $\text{Na}_V1.8$ channels are different in capsaicin responsive neurons than in capsaicin unresponsive neurons. Thus, the heterogeneity of sensory neurons is not simply determined by the relative pattern of expression of different proteins (i.e., see (Matzner and Devor, 1992)), but the properties of these proteins as well. The context of a given protein, in this case $\text{Na}_V1.8$ influences its properties. Thus, $\text{Na}_V1.8$ is subject to use-dependent block, as manifest by a progressive decrease in action potential amplitude in tonic capsaicin responsive neurons. In tonic capsaicin unresponsive neurons, $\text{Na}_V1.8$ is subject to little, if any use-dependent block. An important implication of this observation is that it may not only be possible to selectively block a VGSC, but block a subpopulation of these channels most responsible for persistent pain. A subpopulation of capsaicin responsive neurons thought to play a particularly important role in persistent pain are referred to as silent or sleeping nociceptors (Schmidt, et al., 1995). While highly speculative, a causal link between the use dependent block observed in Yamane's study and the action potential slowing which serves as a defining feature of silent nociceptors, would suggest these afferents may be just the population one would want to selectively target for the greatest therapeutic impact.

Another intriguing aspect of the observations of Yamane and colleagues is that the sensitization of capsaicin responsive neurons, involving mechanisms distinct from $\text{Na}_V1.8$ (i.e., the block of a voltage-gated K^+ channel), conferred therapeutic potential to $\text{Na}_V1.8$. That is, the unique feature of $\text{Na}_V1.8$ channels in capsaicin responsive neurons appeared to be present even if other channels play a more dominant role in limiting the number of action potentials evoked in response to depolarizing stimuli under "normal" circumstances. Block of these channels, as appears to occur in the presence of persistent inflammation (Dang, et al., 2004, Harriott, et al., 2006, Stewart, et al., 2003, Yoshimura and de Groat, 1999), results in an increase in afferent excitability as well as the emergence of $\text{Na}_V1.8$ as an important therapeutic target.

The only perplexing aspect of the Yamane study is the fact that other investigators have not observed the clear distinction between capsaicin responsive and unresponsive neurons with respect to use-dependent block of $\text{Na}_V1.8$ mediated currents induced at stimulation frequencies of 0.3 Hz or higher. In one study, use-dependent block of $\text{Na}_V1.8$ mediated currents was observed in all DRG neurons studied (Blair and Bean, 2002). In others, no use-dependent block was detected in any DRG neuron studied (Brau and Elliott, 1998, Gold and Thut, 2001). In a more recent study, two populations of DRG neurons, one with and one without use-dependent block of $\text{Na}_V1.8$ were described (Choi, et al., 2007). However, in this last study, these two

populations were also defined by the presence or absence of isolectin B4 (IB4) binding rather than capsaicin sensitivity. Use-dependent block of $\text{Na}_V1.8$ was pronounced in IB4+ neurons, and minimal in the IB4- subpopulation. IB4 is a plant lectin that binds to a cell surface carbohydrate enriched in a subpopulation of sensory neurons with a small cell body diameter (Stucky and Lewin, 1999). This subpopulation of nociceptive DRG neurons tends to share a number of other properties including a distinct termination pattern in the superficial dorsal horn of the spinal cord (Braz, et al., 2005), largely unmyelinated axons and the absence of the neuropeptides calcitonin gene related peptide and substance P (Silverman and Kruger, 1988). Importantly, however, multiple lines of evidence suggest that a significant proportion of both IB4+ and IB4- neurons are capsaicin responsive (Dirajlal, et al., 2003, Flake, et al., 2005, Lu, et al., 2006). A possible explanation for the differences between studies is the existence of variability across ganglia. Yamane and colleagues studied neurons from S1 and L6 ganglia (personal communication) because these are more likely to give rise to innervation of visceral structures. Others have studied neurons from L4 and L5 ganglia (Choi, et al., 2007) that are more likely to give rise to innervation of somatic structures. Given several marked differences between visceral and somatic afferents (i.e., see (Gold and Traub, 2004)), it is possible that the distribution of $\text{Na}_V1.8$ with use-dependent block varies according to target of innervation and/or between ganglia. Of course, less interesting explanations like differences in experimental conditions may contribute as well.

Regardless of subpopulation differences, what Yamane and colleagues have again highlighted is the therapeutic potential of a selective blocker of $\text{Na}_V1.8$. That such a compound may have finally been discovered is suggested by a recent description of A-803467, a compound that is more than 100 times more selective for $\text{Na}_V1.8$ than other VGSCs (Jarvis, et al., 2007). Block of $\text{Na}_V1.8$ by A-803467 is state dependent, with inactivated channels blocked at a higher potency than channels in a resting or closed state. Surprisingly, A-803467 did not attenuate postoperative hypersensitivity, serving as a reminder of the importance of context. However, A-803467 did effectively antagonize hypersensitivity associated with nerve injury and persistent inflammation, a therapeutic profile that may still enable an end to a considerable amount of suffering.

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