The response of Na\textsubscript{V}1.3 sodium channels to ramp stimuli: multiple components and mechanisms

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Na\textsubscript{V}1.3 sodium channels are upregulated within dorsal root ganglion (DRG) neurons after axotomy (Black et al. 1999; Waxman et al. 1994) and other forms of peripheral nerve injury (Dib-Hajj et al. 1999) and accumulate within the injured tips of axons within experimental and human neuromas (Black et al. 1999; Black et al. 2008), where ectopic impulses underlying neuropathic pain are generated. Like a number of other sodium channel isoforms, Na\textsubscript{V}1.7 (Cummins et al. 1998), Na\textsubscript{V}1.6 (Herzog et al. 2003), and Na\textsubscript{V}1.3 channels produce a robust depolarizing response to slow ramp-like stimuli (Cummins et al. 2001; Cummins and Waxman 1997). Interestingly, spontaneously firing DRG neurons exhibit slow ramp-like depolarizations during the interspike interval (Estacion et al. 2011; Faber et al. 2012). Na\textsubscript{V}1.3 channels have also been noted to produce a persistent current which is detectable for many tens of milliseconds after the onset of a depolarizing pulse (Chen et al. 2000; Lampert et al. 2006; Sun et al. 2007).

While in many cases the ramp current is TTX sensitive and thus attributable to sodium channels, its underlying basis remains incompletely understood. Multiple mechanisms, including a window current due to the overlap between activation and steady-state inactivation, a slowly inactivating or persistent mode of channel gating, or generation by a separate channel isoform, have all been suggested (Crill 1996; Kiss 2008). In many studies, ramp currents have been elicited and studied in intact neurons, so a contribution from multiple isoforms of sodium channels cannot be excluded.

In this study, we examined the properties of the currents evoked by ramp stimuli and the persistent currents recorded at the end of depolarizing pulses from a single isoform of heterologously expressed sodium channel, Na\textsubscript{V}1.3-WT (wild-type) channels. Using ramps of varying depolarization rates (1.2 mV/ms to 0.2 mV/ms), we could separate the ramp-evoked inward current produced by this single sodium channel isoform into two peaks. We show that the two components of the ramp responses of Na\textsubscript{V}1.3 channels are produced by distinct mechanisms. In comparison, we assess the ramp response of the K354Q Na\textsubscript{V}1.3 mutant channel which was identified in a patient with cryptogenic pediatric partial epilepsy (Holland et al. 2008) and shown by voltage-clamp to increase persistent current (Estacion et al. 2010; Holland et al. 2008). Our results show that a single sodium channel isoform can produce a ramp response with multiple components, reflecting multiple mechanisms, and suggest that the upregulated expression of Na\textsubscript{V}1.3 in axotomized DRG neurons and enhanced ramp current in K354Q mutant channels can contribute in many ways to hyperexcitability disorders, such as epilepsy and neuropathic pain.
were transiently transfected with plasmids containing β1-IRES-CD4 and β2-IRES-GFP (Lossin et al. 2002) and either rNa_{v}1.3_R (referred to as WT hereinafter) or the mutant channel rNa_{v}1.3_R/K354Q (referred to as K354Q hereinafter) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) with a stoichiometry of plasmids of 1:1:5 by mass.

**Electrophysiology.** Whole cell voltage-clamp recordings were obtained as previously described (Estacion et al. 2010) using the following solutions. The extracellular solution contained the following (in mM): 140 NaCl, 3 KCl, 1 MgCl₂, 1 CaCl₂, and 10 HEPES, pH 7.3, with NaOH (adjusted to 320 mosM with dextrose). The pipette solution contained the following (in mM): 135 Cs-aspartate, 10 NaCl, 2 MgCl₂, 0.1 CaCl₂, 11 EGTA (pCa = 8), 10 HEPES, pH 7.2 with CsOH (adjusted to 310 mosM with dextrose). Patch-pipettes had a resistance of 1–3 MΩ when filled with pipette solution. The junction potential of 16 mV (calculated by JCalc, CLAMP software) was compensated by setting holding potential during the seal test period to −16 mV. Once the seal had formed, these two solutions were no longer in contact, and the applied potential was correct. Upon achieving whole cell recording configuration, pipette and cell capacitance were manually minimized using Axopatch 200B (Molecular Devices, Union City, CA) compensation circuitry. To reduce voltage errors, 80–90% series resistance and prediction compensation were applied. Cells were excluded from analysis if the predicted voltage error exceeded 3 mV. Recorded currents were digitized using pCLAMP software (version 10) and a Digidata 1440A interface (Molecular Devices) at 50 kHz after passing through a low-pass Bessel filter setting of 10 kHz. Linear leak and residual capacitance artifacts were subtracted out using the P/4 method (Clampex software). Sodium current recordings were initiated after a 5-min equilibration period once whole cell configuration was achieved.

Data analysis was performed using Clampfit (Molecular Devices) or Origin (Microcal Software, Northampton, MA). To generate activation curves, cells were held at −100 mV and stepped to −80 to +50 mV in 5-mV increments for 100 ms. Peak inward currents from activation protocols were converted to conductance values using the equation, G = I/(V_{m} - E_{Na}), where G is conductance, I is peak inward current, V_{m} is the membrane potential step used to elicit the response, and E_{Na} is the sodium reversal potential (determined for each cell using the x-axis intercept of a linear fit of peak inward current responses). Conductance data were normalized by maximum conductance and fit with a Boltzmann equation of the form G = G_{max} + (G_{max} - G_{min})/[1 + \exp(V_{1/2} - V_{m})/k], where V_{1/2} is the activation midpoint, and k is slope factor. To generate steady-state fast-inactivation curves, cells were conditioned with 500-ms pre-pulses of −170 to −25 mV in 5-mV increments, followed by a 50-ms test pulse to −20 mV. Peak inward currents obtained from steady-state fast inactivation protocol were normalized by maximum current amplitude and fit with a Boltzmann equation of the form I = I_{min} + (I_{max} - I_{min})/[1 + \exp(V_{1/2} - V_{m})/k], where I_{min} represents the inactivating prepulse membrane potential, and V_{1/2} represents the midpoint of inactivation. Persistent current was measured as the average current remaining during the last 10 ms of the 100-ms activation pulses. The ramp stimulus protocol smoothly increases the command potential starting at −100 mV and ending at +20 mV over 600 ms (0.2 mV/ms) and is repeated with incrementally shorter durations to give the indicated ramp rates. The ramp-evoked current traces are post-acquisition filtered from 10 KHz down to 200 Hz to improve data display. Furthermore, nonspecific leak currents indicated by a slope of the ramp traces over the range of −100 mV to −70 mV are removed by performing a linear leak subtraction based on the slope of this region. The amplitude and voltage of the peak inward ramp-evoked currents are identified by a five-point averaging peak finding algorithm between pairs of user-defined cursors placed to bracket the two peaks observed in the ramp traces. Data are expressed as means ± SE. Statistical significance was determined by Student’s t-test.

**RESULTS**

**Voltage-clamp recordings from HEK-293 cells transiently expressing rNa_{v}1.3-WT channels.** Whole cell voltage-clamp recordings were performed on HEK-293 cells transiently expressing WT rNa_{v}1.3_R channels. The current traces elicited in response to the activation protocol of a representative cell are shown in Fig. 1A. The currents elicited from the same cell in response to the fast-inactivation protocol are shown in Fig. 1B. Both sets of traces indicate the presence of persistent current, as defined by inward current remaining at the end of the stimulation pulse (100 ms for activation, 50 ms for fast-inactivation); an expansion of selected traces illustrating the persistent current is shown as an inset to Fig. 1A. The peak inward currents elicited by each stimulus are plotted as a function of the stimulus pulse voltage to generate an activation current vs. voltage (I-V) curve (Fig. 1C) and a fast-inactivation I-V curve (Fig. 1D). The voltage dependence of activation and fast-inactivation was examined by transforming the peak I-V curves into conductance vs. voltage curves, as described in METHODS. The activation and fast-inactivation conductance-voltage curves for this cell are normalized to G_{max} and plotted together in Fig. 1E. The persistent current analyzed from this cell is normalized to peak inward current and also plotted in red in Fig. 1E. The shape and voltage dependence of the persistent current compared with the activation and fast-inactivation curves are shown replotted with an expanded scale in Fig. 1F. Notably, while there is a minor shoulder in the persistent current (arrow) within the voltage range of the window of overlap between activation and steady-state inactivation, the bulk of the persistent current falls outside of the voltage domain of the activation-inactivation window.

Ramp stimulus responses. Most of the Na_{v}1.3-WT-expressing cells exhibited a distinctive double inward peak in response to slow depolarizing ramp protocols, although three of the nine cells displayed only a small first peak, which appeared as a shoulder rather than a well-defined peak. The response of the same cell illustrated in Fig. 1 to ramp stimuli with varying ramp rates is illustrated in Fig. 2A. The ramp-evoked currents clearly show two peaks. The first peak, which is elicited at more hyperpolarized potentials compared with the second peak, is strongly reduced in size by slowing the ramp rate. The shape and voltage at peak for the ramp-evoked current are compared with the persistent current measured for this cell in Fig. 2B. Both the ramp current and the persistent current were normalized to the maximal peak inward current and plotted together for comparison (Fig. 2B). The shape and voltage range for the persistent current do not overlap with the first peak, but correspond more closely to the second peak of the ramp-evoked current.

In the experiment shown in Fig. 2C, the current remaining at the end of the ramp protocol (−100 mV to +20 mV, 600 ms) was exposed to a mirror image slow hyperpolarization or “reverse ramp” (+20 mV to −100 mV, 600 ms) to determine whether either component could recover from inactivation and also to assess whether either component of current deactivates. The reverse ramp current shows that none of peak 1 current remains, suggesting that the peak 1 current does not recover from inactivation during the reverse ramp, while a partial fraction of peak 2 current remains and exhibits deactivation.
Ramp response compared with persistent currents. The relationships between the two peaks of ramp-evoked current are examined in Fig. 3. The values of normalized peak amplitude and ramp voltage for the two peaks derived from the slow (0.2 mV/ms) ramp for a number of individual cells are plotted with a line linking the values corresponding to each individual cell. In Fig. 3A, the distribution of values derived from Na$_{v}$1.3-WT-expressing cells is plotted. The average amplitude for peak 1 is

![Diagram A](image1.png)

![Diagram B](image2.png)

![Diagram C](image3.png)

![Diagram D](image4.png)

![Diagram E](image5.png)

![Diagram F](image6.png)
2.4 ± 0.4%, occurring at −37.3 ± 1.2 mV, and the average amplitude for peak 2 is 5.7 ± 0.9%, occurring at −17.4 ± 1.0 mV (n = 9). The relationship between the normalized amplitude of the second peak of the slow ramp-evoked current and normalized persistent current as measured at the end of sustained depolarizing pulses was evaluated for each of the cells expressing NaV1.3-WT and shown in Fig. 3B. The distribution of points was well described by a straight line, indicating that these two parameters are highly correlated (adjusted $R^2 = 0.98$).

**Comparison to NaV1.3-K354Q mutation.** As a comparison to NaV1.3-WT, we studied the K354Q NaV1.3 mutation, described in a patient with epilepsy (Holland et al. 2008) and shown by voltage-clamp to increase persistent current to 8.1% compared with 4.4% in WT NaV1.3 channels (Estacion et al. 2010). We examined the relationship between the activation and fast-inactivation conductances and persistent current by plotting them together (Fig. 4A) for a selected cell (similar as for a WT cell, Fig. 1E). Similar to NaV1.3-WT cells, the persistent current of K354Q-expressing cells shows only a small shoulder over the voltage range for predicted window current (Fig. 4B, shaded area and arrow) and is much larger at more depolarized membrane voltages. The ramp response of this cell to ramp stimuli with different ramp rates is shown in Fig. 5A, and it too shows two peaks. The first peak again shows a strong decrease as the ramp rate is slowed. The persistent current measured from this cell is plotted against the slow ramp (0.2 mV/ms) response in Fig. 5B. Note the good correspondence of the persistent current and the second peak of the ramp response. As for WT NaV1.3, a reverse ramp-evoked peak 2 current but no discernible peak 1 current from NaV1.3-K354Q-expressing cells (Fig. 5C).

In Fig. 6A, the ramp-evoked peak values derived from NaV1.3-K354Q-expressing cells are plotted. The average amplitude for peak 1 of the current evoked by the slow ramp is 2.6 ± 0.4%, occurring at −38.5 ± 1.4 mV, and the average amplitude for peak 2 is 10.1 ± 2.1%, occurring at −17.2 ± 0.8 mV (n = 12). Consistent with a substantial contribution of persistent current to the second peak of the ramp current, the average normalized peak 2 ramp current of K354Q channels (10.1 ± 2.1%, n = 12) was increased compared with WT-expressing cells (5.7 ± 0.9%, n = 9, P = 0.09). The distribution of points from cells expressing NaV1.3-K354Q mutant also shows a strong correlation between the second peak of the slow ramp-evoked response and the persistent current (Fig. 6B).

Further support for the correlation of the second peak of the slow ramp-evoked current to persistent current is seen when the ramp-evoked currents from both WT and K354Q-expressing cells are normalized and then averaged (Fig. 7). Although on average the first peak smooths into a shoulder on the slow ramp-evoked response, the response of WT and K354Q cells overlays each other during the voltage range of −60 mV through −35 mV. Over the voltage range for the second peak, however, the K354Q ramp response is approximately double that of the ramp response for WT NaV1.3.

**DISCUSSION**

In this study, we examined more closely the properties of the current evoked by slow ramp stimuli from a single sodium channel isoform, NaV1.3. Using ramps of varying depolarization rates (1.2 mV/ms to 0.2 mV/ms) to study NaV1.3-WT channels, we could separate the slow inward currents into two peaks. The first peak showed clear dependence on ramp rate. Since ramp rate determines whether the rate of depolarization is fast enough to activate current before inactivation develops due to depolarization in earlier parts of the ramp, it is likely that the first component of the ramp response is strongly modulated by closed-state inactivation (Cummins et al. 1998). Consistent with this interpretation, the first peak more closely corresponded to the window current predicted by the overlap of the voltage dependence of activation and fast-inactivation. The second peak occurred at more depolarized potentials and appeared to be relatively insensitive to closed-state inactivation. Lampert et al. (2006) demonstrated that NaV1.3 channels can produce a persistent current that is seen as late as tens of milliseconds in activation protocols. Sun et al. (2007) reported that NaV1.3 persistent current is both larger and shifted to more depolarized voltages than the predicted window current. The current density of this second inward ramp current was poorly correlated to transient inward peak. However, consistent with a major contribution of persistent current to the second component of the ramp response, the amplitude of the second ramp component was very well correlated with the current density of persistent current as measured at the end of long depolarization steps. Also suggesting a major contribution of persistent current to the second ramp component, this component of the ramp response was enhanced in the K354Q mutant NaV1.3 channel, which is known (Estacion et al. 2010) to display an enhanced persistent current.

In the literature, reversed ramps have been used to evaluate the persistence and voltage-dependence of deactivation of subthreshold inward currents (Astman et al. 2006; Dai and Jordan 2011; Kononenko et al. 2004; Theiss et al. 2007). If the response to the reverse ramp shows hysteresis, that also indicates continuing slow-inactivation for the subthreshold inward current. The response of NaV1.3 to the reverse ramp indicates that the second ramp component exhibits continued voltage-dependent inactivation, since the magnitude of the second peak is smaller in the reverse direction. The reverse ramp response also indicates a clear voltage-dependent deactivation of the
second peak and reveals a clear loss of current in the voltage range of the first peak.

Magistretti and Alonso (1999) have commented on the definition of persistent sodium current as measured with voltage ramps and note that, depending on ramp speed, multiple kinetic components can be distinguished. Importantly, however, their studies were carried out on dissociated neurons, which likely express multiple isoforms of sodium channels. Whether these components derive from a single channel isoform or from different isoforms with different kinetics is not clear. In our experiments, we were able to distinguish multiple components of the ramp response from a single sodium channel isoform, Na\textsubscript{v}1.3, expressed in a heterologous expression system.

The identity and properties of persistent inward current have been evaluated in many ways. Although persistent inward currents may also be carried by calcium channels (Khaliq and Bean 2010; Perez-Reyes 2003) or non-voltage-gated sodium-permeable channels (Khaliq and Bean 2010), many studies in neurons have implicated voltage-gated sodium channels as contributing to persistent or ramp currents. Some studies, e.g., Del Negro et al. (2002) and Agrawal et al. (2001), refer to ramp responses as persistent currents. Previous studies in intact neurons have demonstrated contributions of window current and persistent current to the ramp response. Consistent with our observations on the first component of the ramp response, Fleidervish and Gutnick (1996) presented evidence indicating that, in neocortical neurons, the ramp-evoked inward current through voltage-gated sodium channels is modulated by closed-state inactivation. However, Magistretti and Alonso (1999), in a study on entorhinal cortex neurons, observed that the current evoked by ramp stimuli had an amplitude and voltage-dependence that could not be accounted for by the window current.

Our description of the two components of the ramp current is not without precedent. For example, in a study on medullary neurons, Rybak et al. (2003) demonstrated two components of ramp current. Many recordings of ramp current from neurons, however, explore the voltage range between \(-80\) mV and about \(-30\) mV (Astman et al. 2006; Theiss et al. 2007), since more depolarized voltages evoke large outward currents as voltage-gated potassium channels activate, confounding direct observation of an additional peak of inward ramp current.

Fig. 2. Na\textsubscript{v}1.3-WT channels exhibit two components of ramp response. A: the currents evoked during a smoothly increasing voltage ramp from \(-100\) mV to \(+20\) mV over 600 ms (black line) are shown from the same Na\textsubscript{v}1.3-WT-expressing cell analyzed in Fig. 1. Ramp response currents recorded using faster ramp rates (see stimulus protocol inset) display a first peak, which occurs at more hyperpolarized potentials and varies in size with ramp rate, decreasing as the ramp rate slows. The second peak is relatively insensitive to ramp rate, consistent with a persistent current. B: superimposition of the persistent current measured at the end of 100-ms stimulus pulses and the slow ramp response (0.2 mV/ms) recorded from the same cell. Both the ramp data and the persistent current data have been normalized to peak current. The filled symbols are the persistent current recorded from this cell (Fig. 1F) using 100-ms stimulus pulses and show correspondence to the second slow ramp peak (gray line, from A, 0.2 mV/ms). C: currents recorded in response to a ramp protocol that reverses direction, as shown in the inset. The current recorded to the rising phase of the ramp stimulus is shown in black, and the current recorded to the falling phase of the ramp stimulus is shown in red. These traces are from a different cell than shown in A, and no leak subtraction was performed on the data traces.
unless the potassium channels are inhibited (Rybak et al. 2003; Zeng et al. 2005). Our results, using heterologous expression into HEK cells which have little endogenous voltage-gated currents, allowed demonstration of two components of ramp current from a single sodium channel isoform, NaV1.3. Further studies will be needed to determine whether other sodium channel isoforms similarly produce ramp currents with multiple components.

It has been suggested (French et al. 1990) that persistent sodium current, including persistent currents evoked by ramp stimuli (Magistretti and Alonso 1999), may be produced by a specialized sodium channel with biophysical characteristics, including late channel (re) openings different from those of the fast transient channels. Our results indicate that a single sodium channel isoform, NaV1.3, can generate at least two mechanically distinct components of ramp-evoked responses, as well as fast-transient current as demonstrated by Cummins et al. (2001). It remains to be determined whether these functionally distinct currents are produced by differentially modulated NaV1.3 channels or by different gating modes of the channel. However, while kinetic gating properties of NaV1.3 are known to be modulated by cell type-specific factors (Chen et al. 2000; Cusdin et al. 2010), it is notable that the three currents can be discerned in a heterologous nonneuronal expression system. Possible mechanisms for the second persistent component of the ramp response include direct modulation of the channels by G protein-mediated pathways (Mantegazzza et al. 2005; Pinet et al. 2008), or cytokine-induced phosphorylation (Binshtok et al. 2008), or by association with

Fig. 3. Average peak amplitude and voltage at peak for NaV1.3-WT inward ramp responses. A: the peak amplitude and the voltage at peak for the two components of the slow ramp (0.2 mV/ms) response are plotted for 9 cells expressing NaV1.3-WT currents. The normalized amplitude and membrane voltage of the two peaks are plotted, and the relationship between the first peak (black circles) and the second peak (red circles) for each cell is indicated by the connecting lines. The average values are shown as colored diamonds with both x and y error bars (SEM). B: there was a good correlation (adjusted $R^2 = 0.98$) between the normalized amplitude of the second peak of the slow ramp response and the normalized persistent current measured for each NaV1.3-WT-expressing cell.

Fig. 4. Voltage-clamp of NaV1.3-K354Q channels. A: the G-V curves for both activation ($V_{1/2} = -33.8$ mV, slope = 7.3, black circles) and fast-inactivation ($V_{1/2} = -74.8$ mV, slope = 6.9, blue squares) for a representative NaV1.3-K354Q-expressing cell obtained by analyzing the activation and fast-inactivation data, as described in Fig. 1. In addition, the normalized persistent current (red diamonds) is also plotted. B: the data in A are replotted with expanded scales to better visualize the relationship between the activation and fast-inactivation curves and the persistent current measured at the end of 100-ms depolarizing pulses.
The formation of an enhanced population of persistent channels by the K354Q mutant, however, suggests the possibility of some other mechanisms, since the mutation substitutes an amino acid within the regulatory β-subunits (Cusdin et al. 2010). The formation of an enhanced population of persistent channels by the K354Q mutant, however, suggests the possibility of some other mechanisms, since the mutation substitutes an amino acid within the

Fig. 5. Na\textsubscript{v}1.3-K354Q channels exhibit two components of ramp response. A: the currents evoked during a smoothly increasing voltage ramp from \(-100\) mV to \(+20\) mV over 600 ms (black line) are shown from the same Na\textsubscript{v}1.3-K354Q-expressing cell analyzed in Fig. 4. Ramp response currents recorded using faster ramp rates (see inset Fig. 2A) indicate that the first peak, which occurs at more hyperpolarized potentials, varies in amplitude with ramp rate, decreasing as the ramp rate slows. The second peak is less sensitive to ramp rate, consistent with a persistent current.

B: the relationship between persistent current measured at the end of 100-ms stimulus pulses is plotted against the slow ramp response (0.2 mV/ms) recorded from the same cell. Both the ramp data and the persistent current data have been normalized to peak current. The filled symbols are the persistent current recorded from this cell (Fig. 4B) using 100-ms stimulus pulses and shows correspondence to the second slow ramp peak (gray line, from A, 0.2 mV/ms). C: currents recorded in response to a ramp protocol that reverses direction as shown in the inset to Fig. 2C. The current recorded to the rising phase of the ramp stimulus is shown in black, and the current recorded to the falling phase of the ramp stimulus is shown in red. These traces are from a different cell than shown in A, and no leak subtraction was performed on the data traces.

Fig. 6. Average peak amplitude and voltage at peak for K354Q inward ramp responses. A: the peak and the voltage at peak for the two components of the slow ramp (0.2 mV/ms) response are plotted for 12 cells expressing Na\textsubscript{v}1.3-K354Q currents. The normalized amplitude and membrane voltage of the two peaks are plotted, and the relationship between the first peak (black circles) and the second peak (red circles) for each cell are indicated by connecting lines. Average values are shown as colored diamonds with both x and y error bars (SEM). B: there was a good correlation (adjusted \(R^2 = 0.88\)) between the normalized amplitude of the second peak of the slow ramp response and the normalized persistent current measured for each Na\textsubscript{v}1.3-K354Q-expressing cell.
extracellular linker between the S5 helix and the pore-loop motif of domain I. The absence of correlation between the fraction of true persistent current and peak inward current suggests that persistent current may not be due to an altered gating scheme for all channels, but is more consistent with the presence of a subpopulation of channels that are able to maintain sustained openings. Parri and Crunelli (1998) modulated the fraction of persistent current by removing fast-inactivation with papan and showed that the voltage-dependence of the newly formed \( I_{\text{Nap}} \) was identical to the \( I_{\text{Nap}} \) found in their neurons.

Our results show that a single sodium channel isoform, \( \text{Na}_{\text{v}}1.3 \), can contribute multiple components to the ramp response. Whether the two components of ramp-evoked inward current play different roles in regulating excitability of neurons is currently unknown. The expression of \( \text{Na}_{\text{v}}1.3 \) within DRG neurons is increased after axonal injury (Black et al. 1999; Dib-Hajj et al. 1999; Waxman et al. 1994). Moreover, \( \text{Na}_{\text{v}}1.3 \) is known to accumulate in the injured tips of axons within experimental (Black et al. 1999) and human neuromas (Black et al. 2008), where ectopic impulses are generated. The more hyperpolarized component of ramp current from \( \text{Na}_{\text{v}}1.3 \) is more likely to be involved in altering threshold. The more depolarized second component of ramp current may, in contrast, play a role in interspike interval pacemaking when neurons or their axons are depolarized after injury. A pharmacological approach to address these hypotheses may be possible, since Sun et al. (2007) report that the concentrations of topiramate required to block \( \text{Na}_{\text{v}}1.3 \) persistent current are much lower than the concentrations required to reduce availability via a drug-induced shift of fast-inactivation voltage dependence. Interestingly, spontaneously firing DRG neurons exhibit interspike interval waveforms which mimic ramp stimuli (Estacion et al. 2011; Faber et al. 2012). Moreover, hippocampal neurons expressing the K354Q Na\textsub{v}1.3 mutant channel, identified in a patient with epilepsy (Holland et al. 2008), display bursts of action potentials with interspike waveforms with a ramp-like trajectory (Estacion et al. 2010). We suggest that the current evoked by slow ramp-like stimuli in WT Na\textsub{v}1.3 channels in axotomized DRG neurons, and by K354Q mutant channels in central neurons, contribute to hyperexcitability, thus playing a role in both neuropathic pain and epilepsy.

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