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$Na_V1.8$ channels are expressed in large, as well as small, diameter sensory afferent neurons

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Sensory neurons in the dorsal root ganglia (DRG) express a subset of voltage dependent sodium channels (Na_V) including $Na_V1.1$, 1.6, 1.7, 1.8 and 1.9. Previous work supported preferential localization of $Na_V1.8$ channels to small-medium diameter, nociceptive afferent neurons. However, we recently published evidence that $Na_V1.8$ was the dominant Na_V channel expressed in the somas of small, medium and large diameter muscle afferent neurons, which is consistent with other reports. Here, we extend those results to show that $Na_V1.8$ expression is not correlated with afferent neuron diameter. Using immunocytochemistry, we found $Na_V1.8$ expression in ~50% of sensory afferent neurons with diameters ranging from 20 to 70 μm. In addition, electrophysiological analysis shows that the kinetic and inactivation properties of $Na_V1.8$ current are invariant with neuron size. These data add further support to the idea that $Na_V1.8$ contributes to the electrical excitability of both nociceptive and non-nociceptive sensory neurons.

Keywords: cutaneous afferents, muscle afferents, dorsal root ganglia neurons, tetrodotoxin-resistant (TTX-R)

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Introduction

$Na_V1.8$ channels are tetrodotoxin-resistant (TTX-R) channels that play a role in action potential generation in the soma of small diameter sensory neurons[1-3] and these channels have been shown to be involved in nociception and chronic pain.[4-9] Thus, the role of $Na_V1.8$ channels in small unmyelinated and thinly myelinated sensory neurons has been well established.[3,10]

However, there is evidence that $Na_V1.8$ channels are also expressed in non-nociceptor sensory afferent neurons. Using both electrophysiology and immunocytochemistry, we recently showed dominant expression of $Na_V1.8$ channels in small, medium and large diameter (> 40 μm) rat muscle afferent neurons.[11] While this was inconsistent with some studies showing minimal expression of $Na_V1.8$ in large diameter cutaneous afferents,[10] it was consistent with other studies using mouse,[12,13] rat[14] and human[15] sensory neurons. Here, we extend our previous results to examine the expression of $Na_V1.8$ channels in sensory neurons, and show that the kinetic properties of these channels do not vary with cell size.

Results

We used immunocytochemistry to assess $Na_V1.8$ expression in sensory afferent neurons isolated from lumbar dorsal root ganglia. The fluorescent intensity and neuronal diameter were measured using ImageJ64 (as previously described).[11] Out of a total of 277 sensory neurons imaged, 140 were stained positive for $Na_V1.8$ (51%) (**Fig. 1A**). The neuronal diameter for $Na_V1.8$ positive neurons ranged from 14–75 μm, while the range for unlabeled neurons was 17–72 μm. The size distribution of $Na_V1.8$ positive neurons showed a peak between 25–35 μm with a reduction of labeled neurons at larger diameters (**Fig. 1B**). However, expressing this histogram as a percentage of $Na_V1.8$ positive neurons showed roughly similar percentages of labeled neurons with diameters

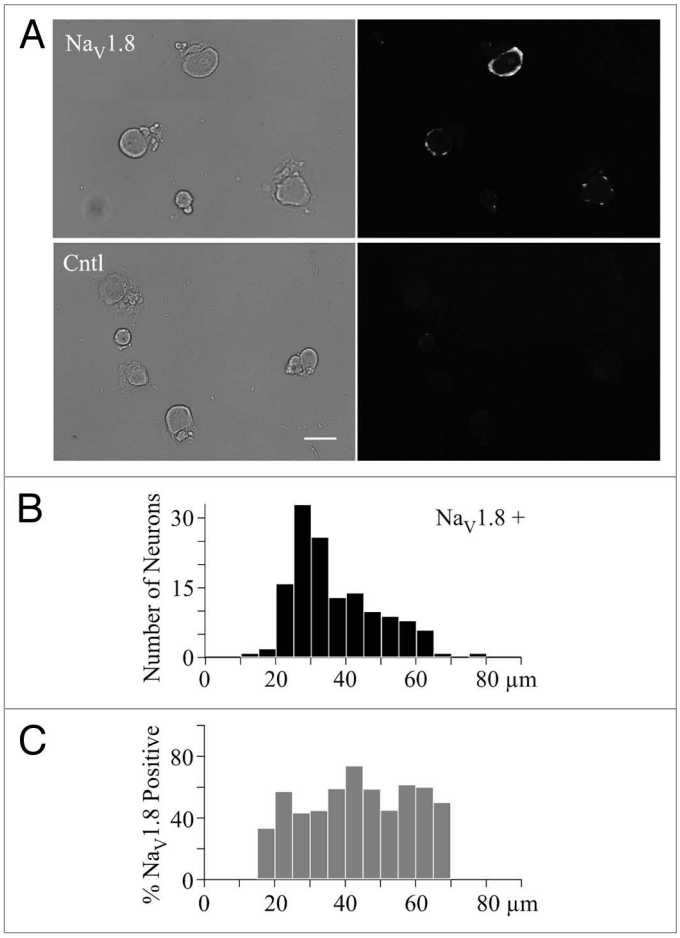

Figure 1. Large diameter sensory neurons express $Na_V1.8$ channels. (**A**) The top row (NaV1.8) shows bright field (left), and $Na_V1.8$ antibody labeling (right) images, while the bottom row (Cntl) shows the bright field (left) and $Na_V1.8$ negative control (right) images. The white bar indicates 50 μm. (**B**) The distribution of $Na_V1.8$ positive ($Na_V1.8$+) sensory neurons vs. cell diameter (5 μm bin width). (**C**) The distribution of percent $Na_V1.8$ positive neurons vs. cell diameter. The 100% values in the 10 and 75 μm bins were removed since there was only a single neuron in each bin. There is a 0% value in the 70 μm bin, which represents data from four neurons.

ranging from 20 to 70 μm (**Fig. 1C**). The percentage of labeled neuron varied between 33–74% (bins with six or more neurons), but there was no clear trend with cell diameter. Thus, there was no preferential labeling of small to medium diameter afferent neurons in this study.

Our previous electrophysiological results from both muscle and cutaneous afferents showed that TTX-R $Na_V1.8$ channels formed the dominant current when the holding potential was –80 mV.[11] However, we did not examine the properties of this current to determine if activation or inactivation properties differed with neuron size. Here we compare the 10–90 activation rise time and inactivation time constant of $Na_V1.8$ current at 10 mV[11] vs. neuronal diameter (**Fig. 2A and B**). The points are clustered around the mean value line regardless of neuron diameter. This was true for both muscle and cutaneous afferent neurons with the caveat that only one large diameter cutaneous afferents was recorded (**Fig. 2A and B**). As a further test of potential differences, we examined the voltage dependence and magnitude of inactivation for the $Na_V1.8$ current in muscle afferent neurons (in 300 nM TTX). The inactivation protocol generated pre- and post-pulses to 10 mV that bracketed a 100 ms inactivating step that ranged from –120 to 30 mV.[16] The postpulse to prepulse current ratio was plotted vs. inactivation voltage and fit using the Boltzmann equation to determine the voltage generating half maximal inactivation (V_{half}) (**Fig. 2C**) and the fraction of total current inactivated (**Fig. 2D**). These data were grouped for small (27–31 μm), medium (31–40 μm) and large (40–50 μm) diameter muscle afferent neurons (**Fig. 2C**), and show that $Na_V1.8$ current inactivation did not vary with neuron diameter, which is consistent with recent experiments using mouse sensory neurons.[13]

Discussion

Previous work showed preferential expression of $Na_V1.8$ channels in small to medium diameter (< 35 μm), nociceptive C and Aδ neurons.[10] Nociceptor expression of these channels has been supported by multiple studies correlating $Na_V1.8$ channel activity with pain.[5,7,9,17-19] While $Na_V1.8$ channels clearly play a role in nociceptor excitability, there is increasing evidence that these channels are functionally expressed in non-nociceptors, including large diameter Aβ sensory afferents that signal vibration sense.[11-13,15] This includes studies from adult human DRG with 60–80% of large diameter neurons (60–80 μm) positively labeled with an $Na_V1.8$ antibody,[15] adult mice with 48% of large neurons positively stained[12] and adult rats with 39% of large DRG neurons positively stained.[14] In addition, studies of skin samples from humans and mice showed $Na_V1.8$ immunoreactivity in primary Aβ afferents innervating cutaneous Meissner's corpuscles and hair cells, which supports $Na_V1.8$ involvement in sensory transduction of fast conducting sensory fibers.[13,15] Here we showed that 33–74% of rat sensory neurons with diameters ranging from 20 to 70 μm were positively labeled by a $Na_V1.8$ antibody. This extends our previous finding that $Na_V1.8$ was the dominant Na_V current in 86% of muscle afferent neurons (25–50 μm) and 12/13 cutaneous afferent neurons (20–50 μm).[11] $Na_V1.8$ channels appear to comprise a large fraction of the active Na_V channels in the soma, and perhaps nerve terminals, of both nociceptive and non-nociceptive sensory neurons.

We also wanted to determine if there were any differences in the $Na_V1.8$ current in these different populations of afferent neurons. Shields et al.[13] recently demonstrated that $Na_V1.8$ activation and inactivation voltage dependent properties

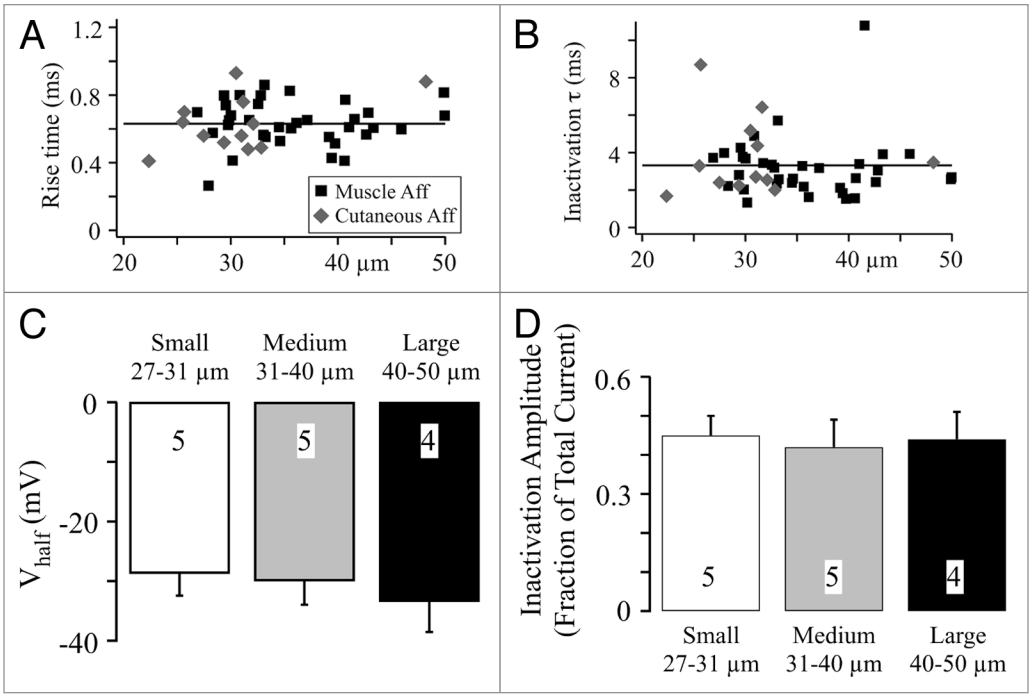

Figure 2. $Na_V1.8$ current properties do not change with sensory neuron diameter. (**A**) Current activation kinetics were measured at 10 mV by the 10–90 rise time[11] and the values are plotted vs. neuron diameter. The solid line represents the average value from all data. (**B**) The inactivation time constant (τ)[11] was measured at 10 mV and is plotted vs. neuron diameter. The solid line and symbols have the same meaning as in (**A**). (**C and D**) V_{half} and maximal inactivation were determined as described in Results. The mean ± SD for small, medium and large sensory neurons is shown. The number of neurons measured is shown in each bar.

were similar between large vs. small DRG mouse neurons. Here we demonstrate that in rat sensory neurons the activation and inactivation kinetics, as well as inactivation voltage dependence and magnitude, of $Na_V1.8$ current are invariant with diameter. While it remains to be tested, $Na_V1.8$ channels may play a similar role in electrogenesis of large diameter afferent neurons as they do in small diameter neurons.[1-3]

Methods and Materials

Sensory neurons were isolated from adult male Sprague Dawley rats obtained from Hill Top Laboratories by enzymatic digestion of lumbar dorsal root ganglia L_4 and L_5.[11] Muscle afferent neurons were identified by retrograde labeling with DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) injected into the right and left triceps surae muscles.[11] All procedures were reviewed and approved by the Institutional Animal Care and Use Committee and followed NIH guidelines.

For immunocytochemistry experiments, isolated DRG neurons were fixed with 4% formaldehyde, permeabilized with 2% Tween 20 and exposed overnight to either the $Na_V1.8$ antibody plus blocking solution (normal goat serum and phosphate buffered saline) (Test) or blocking solution alone (Cntl). The neurons were then washed and exposed to an Alexa Fluor secondary antibody (either Alexa Fluor 350 or 635).[11]

For patch clamp recordings the external solution consisted of (in mM) 45 NaCl, 100 N-methyl d-glucosamine (NMG)•Cl, 4 $MnCl_2$, 10 Na•HEPES and 10 glucose, with pH = 7.4 and osmolarity = 320 mOsm, and the pipet solution contained (in mM) 104 NMG•Cl, 14 Creatine•PO_4, 6 $MgCl_2$, 10 NMG•HEPES, 5 Tris•ATP, 10 NMG_2•EGTA and 0.3 $Tris_2$•GTP with pH 7.4 and osmolarity = 300 mOsm. Neuronal diameter was calculated from membrane capacitance as previously described.[11] Na_V currents were recorded using an Axopatch 200A amplifier and analyzed using Igor Pro (WaveMetrics).

Disclosure of Potential

No potential conflicts of interest were disclosed.

Acknowledgments

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