Sodium Currents in Subthalamic Nucleus Neurons From Na\textsubscript{v}1.6-Null Mice

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Do, Michael Tri H. and Bruce P. Bean. Sodium currents in subthalamic nucleus neurons from Na\textsubscript{v}1.6-null mice. J Neurophysiol 92: 726–733, 2004. First published March 31, 2004; 10.1152/jn.00186.2004. In some central neurons, including cerebellar Purkinje neurons and subthalamic nucleus (STN) neurons, TTX-sensitive sodium channels show unusual gating behavior whereby some channels open transiently during recovery from inactivation. This “resurgent” sodium current is effectively activated immediately after action potential-like waveforms. Earlier work using Purkinje neurons suggested that the great majority of resurgent current originates from Nav 1.6 channels. Here we used a mouse mutant lacking Na\textsubscript{v} 1.6 to explore the contribution of these channels to resurgent, transient, and persistent components of TTX-sensitive sodium current in STN neurons. The resurgent current of STN neurons from Na\textsubscript{v} 1.6\textsuperscript{−/−} mice was reduced by 63% relative to wild-type littermates, a less dramatic reduction than that observed in Purkinje neurons recorded under identical conditions. The transient and persistent currents of Na\textsubscript{v} 1.6\textsuperscript{−/−} STN neurons were reduced by ∼40 and 55%, respectively. The resurgent current present in Na\textsubscript{v} 1.6\textsuperscript{−/−} null STN neurons was similar in voltage dependence to that in wild-type STN and Purkinje neurons, differing only in having somewhat slower decay kinetics. These results show that sodium channels other than Na\textsubscript{v} 1.6 can make resurgent sodium current much like that from Na\textsubscript{v} 1.6 channels.

INTRODUCTION

Among mammalian central neurons there is widespread expression of three types of voltage-dependent sodium channels (Na\textsubscript{v} 1.1, Na\textsubscript{v} 1.2, Na\textsubscript{v} 1.6) that produce TTX-sensitive transient currents, along with more restricted expression of another channel (Na\textsubscript{v} 1.3) with broadly similar characteristics (Caldwell et al. 2000; Felts et al. 1997; Goldin 2001; Schaller and Caldwell 2003). A current challenge is to explore in which expression of particular sodium channels might influence cellular excitability in various neurons.

Na\textsubscript{v} 1.6 channels are present in nodes of Ranvier, unmyelinated axons, cell bodies, dendrites, and presynaptic terminals (Boiko et al. 2001; Caldwell et al. 2000; Krzemien et al. 2000; Schaller and Caldwell 2000, 2003). Mice in which expression of Na\textsubscript{v} 1.6 is eliminated show deficient function of the motor system, including ataxia and progressive paralysis of hind limbs (reviewed by Meisler et al. 2001). Disruption of Na\textsubscript{v} 1.6 expression in mice causes symptoms resembling some human idiopathic dystonias (Hamann et al. 2003). Partial paralysis might be plausibly attributed to disruption of axonal conduction in motor neurons, where Na\textsubscript{v} 1.6 is normally expressed in nodes (Caldwell et al. 2000). Ataxia might be partially accounted for by the widespread expression of Na\textsubscript{v} 1.6 in the cerebellum (Burgess et al. 1995; Schaller and Caldwell 2003). In cerebellar Purkinje neurons of mice that are homozygous for a null allele of Na\textsubscript{v} 1.6, there is a dramatic reduction (80–90%) of an unusual “resurgent” sodium current that flows on repolarization after action-potential-like waveforms (Raman et al. 1997). Loss of Na\textsubscript{v} 1.6 is associated with altered electrophysiological function of Purkinje neurons, including slower and less robust spontaneous firing and reduced burst firing (Khaliq et al. 2003; Raman et al. 1997).

Recent experiments have shown the existence of resurgent sodium current in a number of neuronal types in the motor system in addition to Purkinje neurons, including deep cerebellar nuclei (Raman et al. 2000), subthalamic nucleus neurons (Do and Bean 2003), and globus pallidus neurons (Mercer et al. 2003). Thus it is possible that disrupted function of the motor system in Na\textsubscript{v} 1.6-null mice involves altered function of a number of cell types. To explore this prospect, we examined the consequences of loss of Na\textsubscript{v} 1.6 for sodium current and for the electrophysiological function of subthalamic neurons. We find that without Na\textsubscript{v} 1.6, resurgent current in subthalamic nucleus (STN) neurons is reduced but still present, with only modest changes in kinetic properties or voltage dependence. This adds to other evidence showing that sodium channels in addition to Na\textsubscript{v} 1.6 can make sizeable resurgent sodium current. In addition, loss of Na\textsubscript{v} 1.6 was associated with remarkably little alteration of firing properties of STN neurons, in contrast to the situation in Purkinje neurons.

METHODS

Animals

Heterozygous Scn8a\textsubscript{med} mice were obtained from Jackson Laboratories (Bar Harbor, ME). The med mutation produces complete loss of Na\textsubscript{v} 1.6 expression unlike the med\textsuperscript{+} mutation, which is hypomorphic and results in a reduction in channel expression by ∼90% (Kearney et al. 2002). This had the advantage of totally eliminating Na\textsubscript{v} 1.6 channels but confined our measurements to animals younger than ∼3 wk, when homozygous med mutants die. To compare only homozygous null animals with wild-type animals, we genotyped mice before use and used homozygous med (Na\textsubscript{v} 1.6\textsuperscript{−/−}) or wild-type (Na\textsubscript{v} 1.6\textsuperscript{+/+}) litter-mates. Genotyping used DNA extracted from mouse tails (DNeasy Tissue Kit, Qiagen, Valencia, CA). PCR amplification used the following primers (5’ to 3’): for the wild-type allele, GGA AGG TTC TAG GCA GCT TTA AGT GTG and GTC AAA GCC CGG GAC GTG CAC ACT CAT TCC (Kohrman et al. 1996); for the mutant allele, TCC AAT GCT ATA CCA AAA GTA CC and GGA CGT GCA CAC TCA TTC CC (Jackson Labs). The reaction consisted of 20 s at 94°C, 30 s at 66°C, and 35 s at 72°C (12 repetitions), followed by 20 s at 94°C, 30 s at 60°C, and 35 s at 72°C (25 repetitions), and 5 min at 72°C. PCR products were separated on a 2% agarose gel, allowing resolution of a 230-bp product for the wild-type allele and a 194-bp product for the mutant allele.

Address for reprints and other correspondence: B. P. Bean, Dept. of Neurobiology, Harvard Medical School, 220 Longwood Ave., Boston, MA 02115 (E-mail: bruce_bean@hms.harvard.edu). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Tissue preparation for electrophysiology

In most experiments, the experimenter was blind to genotype and phenotype until after data analysis. Because the ataxia of the mutant animals is generally evident at the ages used (P13-18), another researcher (Dr. Gui-lan Yao) selected animals that had been previously genotyped, anesthetized the animals with isoflurane, and decapitated them. The experimenter received the head, from which the phenotype could not be determined, for further dissection. One or two slices of 300 μm were cut in a cold sucrose solution ([in mM] 87 NaCl, 25 NaHCO3, 2.5 KCl, 1.25 NaHPO4, 7 MgCl2, 25 glucose, and 75 sucrose, equilibrated with 95% O2-5% CO2). Slices were incubated in holding solution (sucrose solution with 0.5 mM CaCl2 added) at 35°C for 30 min then kept at room temperature. For dissociation, slices were exposed to 3 mg/ml protease XXIII for the last 7–8 min at 35°C and then transferred to holding solution with 1 mg/ml BSA and 1 mg/ml trypsin inhibitor during the first 15–30 min at room temperature. The STN was removed into trituration solution ([in mM] 70 NMDG, 1.5 KCl, 7 MgCl2, 6.5 BaCl2, 25 glucose, and 75 sucrose, pH 7.4 with NaOH) and passed through fire-polished Pasteur pipettes to release individual neurons. The STN is well-defined by white matter tracts and contains a highly homogeneous cell population, making cell identification straightforward (Afsharpour 1985; Do and Bean 2003; Song et al. 2000).

For experiments on Purkinje neurons, sections of cerebellar vermis were minced in oxygenated, cold dissociation solution ([in mM] 82 Na2SO4, 30 K2SO4, 5 MgCl2, 10 HEPES, and 10 glucose, 0.001% phenol red, pH 7.4 with NaOH) and transferred to the same solution with 3 mg/ml protease XXIII added. After 7 min at 35°C, tissue was transferred to dissociation solution with 1 mg/ml BSA and 1 mg/ml trypsin inhibitor for 10–15 min at room temperature, then maintained in cold dissociation solution. Tissue was withdrawn as needed and triturated with fire-polished Pasteur pipettes. Purkinje neurons were recognized by their large size and characteristic stump of apical dendrite.

Electrophysiology

Recordings were made using an Axopatch 200B amplifier (Axon Instruments, Union City, CA) in voltage-clamp or fast current-clamp mode. Borosilicate patch pipettes were wrapped to near the tip with Stretch para- medium currents. The internal solution was based on Na2SO4, 30 K2SO4, 5 MgCl2, 10 HEPES, and 10 glucose, 0.001% phenol red, pH 7.4 with NaOH and transferred to the same solution before use so that only homozygous Na v 1.6−/− mice were used. A reduction of 55% in wild-type Na v 1.6+/+ mice (n = 11) and Na v 1.6−/− animals were studied. Figure 1A illustrates the voltage protocol used for eliciting the various components of sodium current. Transient current was measured as fast-activating, fast-inactivating current flowing during a depolarization from −90 to −30 mV. Resurgent current was measured as time-dependent current flowing when the voltage was repolarized to voltages between −80 and −20 mV after a brief (10-ms) depolarization to +30 mV, a protocol that produces maximal resurgent current (Raman and Bean 1997, 2001). Persistent current was measured as the steady-state current flowing at the end of the 100-ms steps after resurgent current had decayed.

Just as in STN neurons from rats (Do and Bean 2003), STN neurons from wild-type mice showed clear resurgent current (18 of 18 cells). Qualitatively, sodium currents in STN neurons from wild-type mice showed clear resurgent current (18 of 18 cells). Qualitatively, sodium currents in STN neurons from wild-type mice showed clear resurgent current (18 of 18 cells). Qualitatively, sodium currents in STN neurons from wild-type mice showed clear resurgent current (18 of 18 cells).

RESULTS

Sodium currents were recorded from STN neurons isolated from med (Na v 1.6−/−) mice and compared with those in wild-type (Na v 1.6+/+) littermates. Animals were genotyped before use so that only homozygous Na v 1.6+/+ or Na v 1.6−/− animals were studied. Figure 1A illustrates the voltage protocol used for eliciting the various components of sodium current. Transient current was measured as fast-activating, fast-inactivating current flowing during a depolarization from −90 to −30 mV. Resurgent current was measured as time-dependent current flowing when the voltage was repolarized to voltages between −80 and −20 mV after a brief (10-ms) depolarization to +30 mV, a protocol that produces maximal resurgent current (Raman and Bean 1997, 2001). Persistent current was measured as the steady-state current flowing at the end of the 100-ms steps after resurgent current had decayed.

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FIG. 1. Resurgent sodium current in subthalamic nucleus (STN) and
Purkinje neurons of wild-type and med mice. A: voltage protocol for
measuring transient, resurgent, and persistent sodium currents. A 20-ms
step to −30 mV from a holding voltage of −90 mV was followed 500 ms
later by a 10-ms conditioning step to +30 mV and a 100-ms test step to
voltages between −20 and −80 mV in 10-mV increments. Each sequence
was separated by 3 s at −90. Below is an example of a family of currents
elicited by the protocol. Transient current was measured as the peak current
from the step to −30 mV minus the steady-state current at the end of the
step. Resurgent current was measured as peak current during the variable
test step minus persistent current, measured as steady-state current at the
end of the test step. B: resurgent current in wild-type and med (Na\textsubscript{v} 1.6\textsuperscript{-/-})
STN and Purkinje neurons. Transient current during the conditioning step
to +30 mV is truncated. The largest resurgent current flowed during a
repolarization to −40 mV in all the examples displayed; decay kinetics are
faster for more hyperpolarized test voltages.

detection in most neurons (Raman et al. 1997). However, the previous experiments were done with a mouse line (med\textsuperscript{38}) where a transgene-induced null allele of Na\textsubscript{v} 1.6 is
maintained in strain C57BL/6J. This is different from the med line used here, where the null allele of Na\textsubscript{v} 1.6 was
caused by insertion of an L1 element and is maintained in a
CH3 background (Sprunger et al. 1999). The previous ex-
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tent internal and external recording solutions, for example
using an external solution with reduced sodium. Thus al-
though the results suggest that resurgent current depends
less on Na\textsubscript{v} 1.6 in STN neurons than in Purkinje neurons, it
is also possible that differences between the mouse strains
or recording conditions complicate the comparison. To eval-
uate this possibility, we performed parallel experiments on
cerebellar Purkinje neurons (Fig. 1B, bottom) from the med
mice and their wild-type littermates using the same solu-
tions as for the STN recordings. Consistent with the earlier
results in the med\textsuperscript{38} mice (Raman et al. 1997), resurgent
current was detected in all wild-type Purkinje neurons
assayed (14 of 14 cells) but was undetectable (5 of 16 cells)
or very small (11 of 16 cells) in med Purkinje neurons.
Transient and persistent sodium currents were also signif-
antly smaller in Purkinje cells from the med mutants.

Examing current density, there was a reduction of 89% for
resurgent current (P = 0.0001), 43% for transient current
(P = 0.002), and 76% for persistent current (P = 0.0004,
Figs. 2 and 3). These findings are very similar quantitatively
to those of Raman et al. (1997), who found that resurgent, transient, and persistent currents in Purkinje neurons of homozygous med\textsuperscript{38} mice were reduced by ~82, 40, and 70% of the levels in wild-type littermates.

The comparison between Purkinje neurons and STN neu-
rons shows that the loss of Na\textsubscript{v} 1.6 reduces transient sodium
current by a similar amount in both cell types (~40%), but
reduces resurgent current more in Purkinje neurons (89%)
than in STN neurons (63%). A straightforward interpreta-
tion is that Na\textsubscript{v} 1.6 sodium channels make up most of the
resurgent current in both cell types but that other sodium
channels can also form resurgent current and do so more
effectively in STN neurons than in Purkinje neurons. Of
course, it is possible that in the mutants there is compensa-
tory upregulation of the sodium currents formed by non-
Na\textsubscript{v} 1.6 channels, in which case Na\textsubscript{v} 1.6 channels in wild-
type animals might make up more than the 40% “missing”
transient current and more than the 89 and 63% “missing”
resurgent current in Purkinje and STN neurons, respect-
ively. Whatever the amount of compensation, two reason-
ably firm conclusions can be drawn with regard to current in
STN neurons: Na\textsubscript{v} 1.6 channels account for the majority of
resurgent current in wild-type animals, but non-Na\textsubscript{v} 1.6
channels can also produce a sizeable resurgent current.

FIG. 2. Quantification of resurgent sodium current in STN and Purkinje
neurons of wild-type and med mice. A, left: histograms of resurgent current
density, measured for a repolarization to −30 mV, are plotted for STN and
Purkinje neurons. Right: histograms of peak resurgent current flowing at −30
mV as a percentage of transient current flowing at −30 mV. B: summary of the
population plots, expressed as means ± SD. *, P ≤ 0.05; **, P = 0.01; and
***, P ≤ 0.001, as evaluated by the Mann-Whitney U test. n = 11 (STN
wild-type and med), 9 (Purkinje wild-type), and 12 (Purkinje med).

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Comparison of resurgent current in Purkinje and STN neurons

The voltage dependence of resurgent current was similar in STN and Purkinje neurons from wild-type and med animals, reaching a peak at $-40$ mV (Fig. 4). However, in both STN and Purkinje neurons from Na$_v$1.6-null mice, the kinetics of both activation and decay of resurgent current were somewhat slower than in wild-type animals. The substantial magnitude of the resurgent current in Na$_v$1.6$^{-/-}$ STN neurons allowed us to compare the resurgent current carried by Na$_v$1.6 channels (which clearly makes up the great majority of the resurgent current in wild-type Purkinje neurons) with that carried by non-Na$_v$1.6 channels, as recorded in Na$_v$1.6$^{-/-}$ STN neurons. After repolarization from $+30$ to $-40$ mV, the resurgent current in Na$_v$1.6$^{-/-}$ STN neurons reached a peak in $5.9 \pm 2.2$ ms ($n = 7$), later than that in wild-type Purkinje neurons ($3.3 \pm 0.4$ ms, $n = 6, P = 0.045$). Resurgent current at $-40$ mV decayed with a time constant of $29 \pm 7$ ms in Na$_v$1.6$^{-/-}$ STN neurons, slower than in wild-type Purkinje neurons, with a time constant of $16 \pm 2$ ms ($P = 0.003$). Thus both activation and deactivation are slower for non-Na$_v$1.6 resurgent current. It is possible that the later time to peak for resurgent current in Na$_v$1.6$^{-/-}$ STN neurons can be accounted for partly by the slower decay even if the intrinsic activation kinetics are not changed, so the primary kinetic difference in Na$_v$1.6$^{-/-}$ neurons may be a decay that is slower by about twofold.

Transient sodium current

Resurgent sodium current appears to arise from an alternative form of inactivation that competes with classical fast inactivation (Raman and Bean 2001). Entry into this second inactivated state can be modeled as an open-channel block, and recovery from it as an voltage-dependent unblock that leaves the channel transiently open, allowing resurgent current to flow. Entry into the inactivated state associated with resurgent current appears to be faster than the competing normal inactivation process, but the normal inactivated state appears to be more absorbing at equilibrium. This predicts a falling phase of transient current that has two components of decay, a fast component reflecting entry into the two inactivated states (initially occurring in parallel) and a slow component of decay arising from the relatively slow redistribution of inactivated channels from the less absorbing to the more absorbing inactivated state; this redistribution is accompanied by transient opening of the channels as the blocking particle leaves, giving rise to the slow component of decay. However, interpretation of the decay kinetics is not straightforward because in addition to two components of decay expected from properties of channels showing resurgence, there could also be multiple components of decay arising from the contribution of multiple channel types with different speeds of inactivation, and even individual channel types can have conventional inactivation with decay described by two time constants (Spampanato et al. 2001).

Figure 5 illustrates the kinetics of transient current in wild-type and med STN and Purkinje neurons. Transient sodium current was evoked by a step to $-30$ mV from a holding potential of $-90$ mV. In all cases, the time course of decay could be reasonably well fit by the sum of two exponentials. There was no clear difference in the kinetics of transient current between wild-type and med STN neurons; in both cases, the fast time constant was $\sim 0.5-0.6$ ms ($P = 0.6$), the
Firing properties of STN neurons lacking Na\textsubscript{1.6}.

The firing properties of Purkinje neurons lacking Na\textsubscript{1.6} are disrupted, with a reduction in pacemaking frequency (Khaliq et al. 2003) and reduced (though not eliminated) tendency to fire bursts of spikes in response to brief injections of currents (Raman et al. 1997). To test how the loss of Na\textsubscript{1.6} affects the physiology of med STN neurons, we performed a series of current-clamp experiments in the brain-slice preparation. As expected from previous recordings from rat STN neurons in brain slice (Beurrier et al. 1999; Bevan and Wilson 1999; Do and Bean 2003), mouse STN neurons showed rhythmic spontaneous firing in the absence of current injection. The STN neurons of med as well as wild-type mice showed spontaneous activity. On average, there was no clear difference in the frequency of firing in med compared with wild-type mice, although individual neurons varied widely in their frequency of firing. The frequency of firing was 12 ± 9 Hz (n = 10) in wild-type neurons and 12 ± 5 Hz (n = 16, P > 0.5) in med neurons. We also examined firing of STN neurons when driven by current injection (Fig. 6). In these experiments, too, there was surprisingly little difference between med and wild-type neurons. There was no clear difference in the frequency of firing in response to injections of increasing amounts of current (Fig. 6B). The only noticeable and reproducible difference in firing behavior was in the tendency to fire action potentials after the cessation of current injection for large stimuli. Most wild-type neurons fired a burst of action potentials during repolarization after a strong stimulus (asterisks, Fig. 6A). Of 15 wild-type neurons studied, 11 fired at least one spike after interruption of the simulating current pulse, and 9 fired at least two spikes, and 5 fired five or more spikes. Of 11 med neurons studied, 5 fired at least one spike, 2 fired at least two spikes, and none fired more than four spikes. Thus the reduction of sodium current in med STN neurons does impact the firing properties of these cells, but in a relatively subtle manner.

**DISCUSSION**

We found that sizeable resurgent currents were still present in STN neurons from Na\textsubscript{1.6}-null mice. Although this was unexpected given the dramatic reduction in resurgent current...
seen previously in Purkinje neurons (Raman et al. 1997)—confirmed by our own measurements in a different null mutant—it adds to accumulating evidence that sodium channels other than Na\textsubscript{v}1.6 can form resurgent current. Although resurgent sodium current in Na\textsubscript{v}1.6-null Purkinje neurons was very small when initially assayed (Raman et al. 1997) with 50 mM external sodium, used to improve voltage control, it was clearly present in some neurons, and its presence is more obvious and unambiguous when studied with physiological solutions (present results; T. M. Grieco and I. M. Raman, personal communication). Additionally, even in individual Purkinje neurons from Na\textsubscript{v}1.6-null mice where resurgent current is undetectable, resurgent current can be induced if the remaining sodium channels, most likely Nav1.1 channels (Schaller and Caldwell 2003; Vega-Saenz de Miera 1997), are treated with beta-pom pilido toxin, which slows inactivation (Grieco and Raman 2004). The simplest interpretation of these results is that the open-channel-blocking mechanism underlying resurgent current can operate with all sodium channel types and is normally more prominent with Na\textsubscript{v}1.6 channels, probably because in those channels this mechanism competes more effectively with conventional inactivation (Grieco and Raman 2004), thought to be due to block of channels by the domain III-IV linker (Catterall 2000). Conversely, resurgent current was not detected in some cell types that clearly express Na\textsubscript{v}1.6, including CA3 pyramidal neurons and motor neurons (Garcia et al. 1998; Pan and Beam 1999; Raman and Bean 1997).

While showing that channels other than Na\textsubscript{v}1.6 can form resurgent current, our results add to previous results in suggesting that Na\textsubscript{v}1.6 channels are more effective than other sodium channel types in forming resurgent current. In our results with STN neurons, loss of Na\textsubscript{v}1.6 had a substantially larger effect in reducing resurgent current (by 63%) than transient current (by 40%). Similarly, loss of Na\textsubscript{v}1.6 reduced persistent current (by 55%) somewhat more than transient current in STN neurons. This is similar to previous results for a disproportionate reduction of persistent current in both Purkinje neurons (Raman et al. 1997) and prefrontal cortex pyramidal neurons (Maurice et al. 2001) from Na\textsubscript{v}1.6-null mice, and the results are also consistent with data from heterologous expression studies showing that Na\textsubscript{v}1.6 channels are more effective than Na\textsubscript{v}1.1 and Na\textsubscript{v}1.2 channels in producing persistent current (Smith et al. 1998). In the heterologous expression studies, Na\textsubscript{v}1.6 channels did not produce clear resurgent current, presumably because the hypothetical blocking particle is lacking.

It is notable that persistent current is substantially larger in relation to transient current in wild-type Purkinje neurons (ratio of persistent current to transient current 2.7%, Raman et al. 1997; 1.7%, this study) than in wild-type STN neurons (0.6%) or Na\textsubscript{v}1.6-null STN neurons (0.6%). The simplest possibility is that Na\textsubscript{v}1.6 channels in Purkinje neurons have conventional inactivation that is less complete than for Na\textsubscript{v}1.6 channels in STN neurons or for Na\textsubscript{v}1.1 or Na\textsubscript{v}1.2 channels. Differences between Na\textsubscript{v}1.6 channels in different types of neurons could reflect expression of different accessory beta subunits, which affect channel gating (Qu et al. 2001). In addition, regulation of native resurgent current by phosphorylation (Grieco et al. 2002) and of inactivation of heterologously expressed Na\textsubscript{v}1.6 channels by calcium/calmodulin (Herzog et al. 2003) have both been reported, and either of these mechanisms could underlie functional differences in currents formed by Na\textsubscript{v}1.6 channels in different cells. In principle, the presence of the blocking particle hypothesized to underlie resurgent current might result in less steady-state persistent current than would otherwise be the case; however, detailed modeling of resurgent current suggests that block by this particle is steeply voltage dependent (Raman and Bean 2001) and is weak over the voltage range where persistent current is prominent (~60 to ~30 mV). Thus there is no fundamental inconsistency between the presence of a large resurgent current and a large persistent sodium current.

If the conventional inactivation of Na\textsubscript{v}1.6 channels in Purkinje neurons is less complete than that of Na\textsubscript{v}1.6 channels in STN neurons, the channels in Purkinje neurons also would be predicted to produce a larger resurgent current relative to transient current (because a greater fraction of channels can be blocked by the “resurgent particle” in competition with conventional inactivation). This idea is consistent with transient current being reduced ~40% in both Purkinje and STN neurons that lack Na\textsubscript{v}1.6 but resurgent current being reduced much more in Purkinje neurons than in STN neurons.

Resurgent current in STN neurons is faster to rise and faster to decay with Na\textsubscript{v}1.6 present. Such a difference would be expected if the hypothetical blocking particle binds more weakly to Na\textsubscript{v}1.6 channels than non-Na\textsubscript{v}1.6 channels. More rapid unbinding of the particle from channels during repolarization would result in a faster rise of resurgent current, and biasing the equilibrium between open and blocked states in favor of open states would result in a faster decay of resurgent current because channels are delivered more quickly to conventional inactivated states. Such a kinetic difference also would tend to produce a larger peak resurgent current and may account in part for the greater effectiveness of Na\textsubscript{v}1.6 channels in producing such current. However, the most important factor in this regard is likely to be a slower rate of conventional inactivation in Na\textsubscript{v}1.6 channels than non-Na\textsubscript{v}1.6 channels (at least during strong depolarizations), allowing a larger fraction of channels to bind the “resurgent” blocking particle before undergoing conventional inactivation (Grieco and Raman 2004).

In principle, a reduction in the magnitude of resurgent current relative to transient current (as seen in both STN and Purkinje neurons) might be expected to be accompanied by a reduction in the magnitude of the slow phase of decay of transient current. In fact, however, the relative contribution of the slow time constant of inactivation showed little change in med compared with wild-type mice in either STN or Purkinje neurons (while the speed of decay of this component changed in med Purkinje cells but not STN neurons). Interpretation of the kinetics of overall transient current is difficult because it reflects the combined current of all the sodium channels present, including any that do not form resurgent current. Even a single-channel type with only conventional activation can have inactivation kinetics with two time constants (Spampanato et al. 2001). Thus although the decay kinetics of overall transient current could be fit reasonably well by two time constants, the slow time constant probably reflects not just the unbinding of the “resurgent” blocking particle but also conventional inactivation of a complex mixture of multiple channel types, some of which may be upregulated in response to loss of Na\textsubscript{v}1.6.
It was surprising that the firing properties of STN neurons studied in brain slice were so little different in homozygous med mutants compared with wild-type littermates. Neither the frequency of spontaneous firing nor the frequency of firing in response to current injection were different. The only clear difference was a reduction of burst firing after switching off of large current injections that induced depolarization block; this reduction of burst firing in STN neurons is reminiscent of a reduction in all-or-none burst firing (fewer spikes per burst stimulated with brief current injections) seen in Purkinje neurons from Na\(_{\text{v}}\),1.6-null mice (Raman et al. 1997). Resurgent current immediately after a spike is expected to promote firing of a subsequent spike, both as a result of the inward resurgent current itself and because the flow of this current appears to be associated with rapid recovery from inactivation (Raman and Bean 2001; Raman et al. 1997). The number of spikes in a burst may be very sensitive to the level of resurgent current since spike success or failure depends on whether the sum of a number of large currents following a spike is barely net inward or barely net outward (Swensen and Bean 2003).

Previous work in Purkinje and STN neurons has suggested that resurgent current also helps generate the rapid spontaneous firing that is typical of these cell types (Do and Bean 2003; Khaliq et al. 2003), so a reduction in resurgent current by an average of 63% would be expected to result in a significantly lower frequency of firing if there were no compensatory changes in other currents. However, it is very plausible that other currents do change in the absence of Na\(_{\text{v}}\),1.6 in a manner to favor faster pacemaking. In Purkinje neurons from med mice, Khaliq and colleagues (2003) showed that the voltage dependence of a component of potassium current is altered so as to be less readily activated (midpoint shifted in the depolarizing direction) and that input resistance of the cells is increased; their modeling showed that both changes tend to promote faster firing. There is also an upregulation in the magnitude of both T- and P-type calcium currents in Purkinje neurons of med mice (A. Swensen and B. P. Bean, unpublished results). If similar changes in input resistance, voltage-activated potassium currents, or calcium currents occur in STN neurons, they could produce faster pacemaking than would otherwise be the case.

Another possibility relates to the fact that the current-clamp experiments were done with STN neurons in brain slice, while changes in sodium current under voltage clamp were studied in acutely dissociated cell bodies (necessary to ensure adequate voltage control). In both Purkinje neurons and retinal ganglion neurons, Na\(_{\text{v}}\),1.6 channels are clustered at high density at the initial segment of the axon by a mechanism involving ankryin-G (Boiko et al. 2003; Jenkins and Bennett 2001), and in layer 5 pyramidal neurons and subicular neurons, spike formation occurs first in the axon, ≳30 \(\mu\)m away from the soma (Colbert and Johnson 1996; Stuart et al. 1997). There is also evidence that axonal channels have different voltage dependence, requiring smaller depolarizations for activation (Colbert and Pan 2002). Thus the current-clamp behavior of cells in brain slice may depend most strongly on the properties and density of sodium channels in the axon. Previous experiments relating changes in sodium current to changes in firing characteristics of Purkinje neurons used dissociated neurons for current-clamp as well as voltage-clamp experiments, allowing direct comparison between the two sets of measurements.

Unfortunately, we were unable to obtain good current-clamp recordings from STN neurons dissociated from med mice; the cells generally were not robust enough to sustain spontaneous activity (unlike their behavior when studied in brain slice), so it was not feasible to examine the current-clamp properties in dissociated neurons.

Whatever the explanation for the relatively minor changes in current-clamp behavior of STN neurons from med mice, the experiments suggest that the functional role of STN neurons may be considerably less affected by loss of Na\(_{\text{v}}\),1.6 channels than that of Purkinje neurons and that changes in STN neuron function probably play little role in the motor deficits of Na\(_{\text{v}}\),1.6-null mice. In this regard, an important limitation of our study is that expression of Na channels changes during development, and our analysis was of necessity confined to relatively young animals. Na\(_{\text{v}}\),1.6-null animals die at ≃3 wk, probably in connection to the replacement of Nav1.2 by Nav1.6 that normally occurs at this time in nodes of Ranvier (Boiko et al. 2001). Thus it is possible that in fully mature mice, elimination of Na\(_{\text{v}}\),1.6 channels would have more dramatic consequences for the function of STN neurons, especially if this could be studied in the absence of compensatory changes in expression of other channels.

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