Similar Properties of Transient, Persistent, and Resurgent Na Currents
in GABAergic and Non-GABAergic Vestibular Nucleus Neurons

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Gittis AH, du Lac S. Similar properties of transient, persistent, and resurgent Na currents in GABAergic and non-GABAergic vestibular nucleus neurons. J Neurophysiol 99: 2060–2065, 2008. First published February 20, 2008; doi:10.1152/jn.01389.2007. Sodium currents in fast firing neurons are tuned to support sustained firing rates >50–60 Hz. This is typically accomplished with fast channel kinetics and the ability to minimize the accumulation of Na channels into inactivated states. Neurons in the medial vestibular nuclei (MVN) can fire at exceptionally high rates, but their Na currents have never been characterized. In this study, Na current kinetics and voltage-dependent properties were compared in two classes of MVN neurons with distinct firing properties. Non-GABAergic neurons (fluorescently labeled in YFP-16 transgenic mice) have action potentials with faster rise and fall kinetics and sustain higher firing rates than GABAergic neurons (fluorescently labeled in GIN transgenic mice). A previous study showed that these neurons express a differential balance of K currents. To determine whether the Na currents in these two populations were different, their kinetics and voltage-dependent properties were measured in acutely dissociated neurons from 24- to 40-day-old mice. All neurons expressed persistent Na currents and large transient Na currents with resurgent kinetics tuned for fast firing. No differences were found between the Na currents expressed in GABAergic and non-GABAergic MVN neurons, suggesting that differences in properties of these neurons are tuned by their K currents.

INTRODUCTION

Different firing properties of neurons are established by the tuning of ionic currents, apparent at the molecular level in the diversity of potassium (K) channel and sodium (Na) channel expression across cell types (Catterall et al. 2005; Coetzee et al. 1999). Historically, the kinetic properties of voltage-gated Na channels were considered to be relatively homogeneous, but more recently, important differences in their kinetic and voltage-dependent properties have been identified across cell types with different firing properties (reviewed in Bean 2007). For example, Na currents in regular spiking hippocampal neurons have different inactivation kinetics and voltage dependencies than Na currents in fast firing interneurons in the hippocampus (Martina and Jonas 1997) and Purkinje cells in the cerebellum (Raman and Bean 1997).

During firing, Na current availability is limited by the accumulation of Na channels into fast (Armstrong 1981; Stuhmer et al. 1989; Vassilev et al. 1988) and slow inactivated states (Mitrovic et al. 2000; Ong et al. 2000; Ulbricht 2005). Na currents in fast firing neurons are slower to enter and faster to recover from these inactivated states than Na currents in slower firing neurons (Martina and Jonas 1997). Additionally, Na currents in many fast firing neurons are protected from inactivation by an endogenous blocking particle that competes for position in a Na-channel pore with the channel’s inactivation gate (Grieco et al. 2005; Raman and Bean 1997). This mechanism is revealed in voltage-clamp experiments by the presence of a resurgent Na current (Afshari et al. 2004; Aman and Raman 2007; Baufreton et al. 2005; Do and Bean 2003; Raman and Bean 1997; Raman et al. 2000).

Neurons in the medial vestibular nuclei (MVN) rapidly encode changes in head and image motion with linear changes in firing rate and can sustain firing rates up to hundreds of Hz when depolarized with current injection. In contrast to the cellular framework in the hippocampus and cortex, both GABAergic and non-GABAergic neurons in the MVN are fast firing, and non-GABAergic neurons have narrower action potentials and higher maximum firing rates than GABAergic neurons (Bagnall et al. 2007). Although GABAergic and non-GABAergic MVN neurons express a different balance of K currents that contributes to differences in their action potential repolarization (Gittis and du Lac 2007), the properties of their Na currents have never been characterized.

Neurons from the same structure with different firing properties often express Na currents with distinct properties (Afshari et al. 2004; Aman and Raman 2007; Martina and Jonas 1997). To determine whether Na currents are differentially tuned between GABAergic and non-GABAergic MVN neurons, the amplitude and kinetic properties of Na currents, including persistent and resurgent currents, were measured from acutely dissociated MVN neurons. The results of this study show that Na currents in MVN neurons are similarly tuned to support fast firing in both GABAergic and non-GABAergic MVN neurons.

METHODS

Cell preparation

Coronal slices (350 μM) through the rostral 2/3 of the MVN were prepared with a DSK-1500E or Leica VT1000S Vibratome in carbogenated artificial cerebrospinal fluid containing (in mM) 125 NaCl, 26 NaCHO3, 5 KCl, 1.3 MgCl2, 2.5 CaCl2, 1 Na2HPO4, and 11 glucose. Slices were heated for 10–30 min at 34°C then maintained at room temperature. Neurons were enzymatically dissociated, as described in Gittis and du Lac (2007), from 24- to 40-day-old mice, either GIN (Oliva et al. 2000) for GABAergic neurons or YFP-16 (Feng et al. 2000) for non-GABAergic neurons, both in c57BL/6 backgrounds. Briefly, slices were treated with 40 U/mL papain (Worthington) in 9.4
Electrophysiological recording

For the duration of a recording session (2–3 h), neurons were continuously perfused with oxygenated Tyrode’s solution (in mM: 150 NaCl, 3.5 KCl, 2 CaCl2, 1 MgCl2, 10 Heps, 10 glucose) and all recordings were done at room temperature. Whole cell recordings were made with borosilicate pipettes (2–4 MΩ), filled with a K-glucuronate-based intracellular solution in mM: 140 K-glucuronate, 8 NaCl, 10 Heps, 0.02 EGTA, 2 Mg-ATP, 0.3 Na2-GTP, and 14 Tris-creatine PO4.

The measured liquid junction potential was +15 mV and was corrected off-line. Sodium currents were isolated by digital subtraction following application of 1 μM tetrodotoxin (TTX; Tocris) in the presence of Tyrode’s solution containing 20 mM tetraethylammonium, 5 mM 4-aminopyridine, and 2 mM MgCl2 substituted for 2 mM CaCl2 (Sigma).

Data were collected and analyzed using IGOR software with a MultiClamp 700B amplifier (Axon Instruments) and an ITC-16 interface (InstruTECH). Ionic currents were recorded in voltage-clamp mode, filtered at 8 kHz, and digitized at 40 kHz. Whole cell capacitance was compensated through the amplifier circuitry and series resistance (Rs) was compensated at 70–90%. The average uncompensated series resistance was 1.9 ± 1.0 MΩ (n = 25) in GABAergic and 1.6 ± 0.8 MΩ (n = 25) in non-GABAergic neurons (P = 0.27). The capacitance was measured in the unclamped amplifier or by integrating the area of the transient following a step from −65 to −75 mV with whole cell capacitance and series resistance compensation turned off. Average cell capacitance was 7.2 ± 2.8 pF (n = 25) for GABAergic and 8.5 ± 3.5 pF (n = 25) for non-GABAergic neurons (P = 0.15).

Data analysis

Na conductance (gNa) was calculated with the equation gNa = \( g_{\text{Na}} = \frac{I(V - E_{\text{Na}})}{e} \), where \( E_{\text{Na}} \) was calculated to be +46 mV in 50 mM NaCl at 22°C. Na conductance at each voltage was normalized to the maximum conductance (usually at −25 mV in 50 mM NaCl) in each cell to create a normalized conductance plot. The normalized conductance plot in each neuron was fit with a Boltzmann equation: \( g_{\text{max}} / (1 + \exp(V_{1/2} - V/k)) \), where \( g_{\text{max}} \) is the normalized maximum conductance, \( V_{1/2} \) is the voltage at which half of the channels are open or closed, \( V \) is the voltage at which conductance was calculated, and \( k \) is the slope, a measure of the voltage dependence of the channels.

To calculate the voltage dependence of fast inactivation, Na current availability was measured at +15 mV after 100-ms voltage steps to potentials between −85 and 0 mV. Availability after the 100-ms voltage steps was assessed by normalizing the amplitude of the noninactivated current by the amplitude of the noninactivated current after the 100-ms voltage step to −85 mV.

Statistical differences were tested with the nonparametric Wilcoxon test for unpaired data and variances reported in the text are SDs.

**RESULTS**

**Transient Na current**

To determine whether Na currents were differentially tuned in GABAergic versus non-GABAergic MVN neurons, transient Na currents were recorded from fluorescently labeled neurons, acutely dissociated from lines of mice that label GABAergic (GIN) or non-GABAergic (YFP-16) neurons in the MVN (Bagnall et al. 2007). Transient Na currents were elicited with 20-ms voltage steps to positive potentials from a holding potential of −80 mV. The amplitudes of transient Na currents in non-GABAergic neurons were larger than those in GABAergic neurons (13.8 ± 4.5 nA, n = 24 vs. 10.8 ± 4.7 nA, n = 22, P = 0.04) but their current densities were similar (1.9 ± 0.7 vs. 1.8 ± 0.9 nA/pF, P = 0.47), suggesting their different current amplitudes reflected larger surface areas of non-GABAergic neurons (Bagnall et al. 2007) rather than differences in Na current expression.

Activation of the transient Na current at 0 mV was complete within 0.34 ± 0.04 ms in GABAergic (n = 13) and 0.31 ± 0.04 ms in non-GABAergic (n = 15) neurons (P = 0.24). To measure the voltage dependence of activation, a family of voltage steps was delivered in a low Na external solution (50 mM) to reduce the current amplitude and minimize \( R_s \) errors (Fig. 1A). Even in 50 mM NaCl, the maximum transient Na current was often >6 nA, creating 8- to 12-mV deviations between the command potential of the amplifier and the voltage experienced by the cell. To decrease these errors, the peak Na current was further reduced to <3.5 nA with saturating concentrations of TTX. This allowed for accurate measurement of the voltage dependence of activation, but precluded measurements of true maximum conductance. As a result, normalized conductance plots were compared across neurons (see METHODS). In both cell types, Na currents began to activate around −55 mV and were fully activated by about −5 mV (Fig. 1C). Boltzmann fits from individual neurons to determine the voltage of half-maximal activation (\( V_{1/2} \)) and the slope (\( k \)) yielded no differences between the cell types (\( V_{1/2} \), P = 1; \( k \), P = 0.89, n = 11 GABAergic and 10 non-GABAergic).

To measure the kinetics of inactivation, the decay of transient Na currents at 0 mV (in 150 mM NaCl) were fit with a single exponential. The \( \tau \) of fast inactivation was the same in GABAergic (0.34 ± 0.03 ms, n = 13) and non-GABAergic neurons (0.34 ± 0.05 ms, n = 15) (P = 0.96). To compare the voltage dependence of inactivation, Na channel availability at +15 mV was assessed following a 100-ms prestep to different potentials (Fig. 1B). Na channels began to inactivate during 100-ms steps to −70 mV in both cell types and were fully inactivated by 100-ms steps to −35 mV (Fig. 1C). Boltzmann fits of the steady-state inactivation curves from individual cells yielded no differences in \( V_{1/2} \) or \( k \) (\( V_{1/2} \), P = 0.28; \( k \), P = 1) between GABAergic (n = 12) and non-GABAergic neurons (n = 11).

**Na current inactivation and recovery**

The time courses of recovery of transient Na currents in GABAergic and non-GABAergic MVN neurons were compared following either a 2- or a 500-ms step to 0 mV to drive Na channels into fast and slow inactivated states, respectively. Recovery from inactivation was assessed by measuring the Na current availability 1 ms to 1 s after the initial depolarizing step (Fig. 2A).

In both GABAergic and non-GABAergic neurons, transient Na currents were initially reduced to 41% following a 2-ms step to 0 mV (P = 0.86). Within 10 ms, >80% of the Na current had recovered. Recovery followed a double-exponential time course with similar time constants for both GABAergic (1.7 ± 0.3 and 138 ± 38 ms, n = 12) and non-GABAergic neurons.
neurons (1.7 ± 0.4 and 117 ± 58 ms, n = 11) (τ₂ = 0.64; τ₂ = 0.01) (Fig. 2B).

Following a 500-ms voltage step to 0 mV, transient Na currents were reduced to 10–13% of their initial value in both GABAergic and non-GABAergic neurons (P = 0.25) (Fig. 2B). Recovery was considerably slower, requiring almost 500 ms for 80% recovery, and followed a double-exponential time course with similar time constants for GABAergic (3.6 ± 1.6 and 248 ± 103 ms, n = 12) and non-GABAergic neurons (3.9 ± 2.1 and 220 ± 134 ms, n = 11) (τ₁ = 0.97; τ₂ = 0.25) (Fig. 2B).

To test for cell type differences in the rate of entry into the slow inactivated state, neurons were held at 0 mV for durations of 1 ms to 2.5 s and Na channel availability was assessed after 75 ms at −80 mV to relieve fast inactivation (Fig. 2C). Slow inactivation in both GABAergic and non-GABAergic MVN neurons took almost 100 ms to accumulate at 0 mV and followed a similar time course in both cell types (Fig. 2D). Although τ₁ was significantly shorter in GABAergic neurons (60 ± 41 vs. 121 ± 53 ms, P = 0.01), there were no differences in the dominant time constant (τ₂) between the cell types (GABAergic: 1.125 ± 415 ms, n = 12; non-GABAergic: 1.360 ± 489 ms, n = 8) (Fig. 2D).

**Persistent Na current**

Persistent Na currents are present in diverse cell types (Crill 1996) and can contribute to autonomous pacemaking (Bevan and Wilson 1999; Do and Bean 2003; Raman and Bean 1999; Shao et al. 2006; Taddeese and Bean 2002) or burst firing (Del Negro et al. 2002; Enomoto et al. 2006; Wu et al. 2005). To isolate persistent Na currents in MVN neurons, cells were slowly depolarized with a 1-s ramp stimulus from −95 to +20 mV (115 mV/s), before and after application of 1 μM TTX (Fig. 3A). Both cell types expressed a persistent Na current between −70 and −20 mV and there was no difference in the maximum amplitude or voltage dependence of the current between GABAergic (233 ± 113 pA, n = 11) and non-GABAergic neurons (232 ± 148 pA, n = 11; P = 0.95) (Fig. 3B).

**Resurgent Na current**

To measure resurgent Na currents in MVN neurons, cells were held at 0 mV for 10 ms, then repolarized to voltages between −60 and −10 mV (Fig. 4A). At these voltages, a “resurgent” Na current flows through channels that were protected from inactivation during the 10-ms depolarization step, possibly by a peptide-blocking particle (Grieco et al. 2005; Raman and Bean 1997). In both GABAergic and non-GABAergic MVN neurons, resurgent Na currents were largest at −35 mV and were, 13 ± 3 and 15 ± 5% of the transient Na current (measured at 0 mV) respectively. At −35 mV, the resurgent current reached its peak amplitude within 2.8 ± 0.6 ms (n = 13) in GABAergic and 2.9 ± 0.4 ms (n = 14) in non-GABAergic neurons (P = 0.54) and decayed exponentially with a time constant of 9.6 ± 1.3 ms in GABAergic and 10.8 ± 1.9 ms in non-GABAergic neurons (P = 0.13). The resurgent Na current was not significantly different in non-GABAergic neurons (−797 ± 304 pA, n = 14) compared with GABAergic neurons (−697 ± 282, n = 13) (P = 0.23) (Fig. 4B).

**DISCUSSION**

This study is the first to describe the biophysical properties of Na currents in MVN neurons which are capable of firing at exceptionally high rates. All MVN neurons had persistent Na currents as well as large transient Na currents with resurgent kinetics and voltage dependencies similar to those of other fast firing neurons. A comparison of the Na currents expressed in two classes of MVN neurons with different firing properties, GABAergic and non-GABAergic neurons, revealed that their Na currents were very similar. This finding was surprising given that neurons with different firing properties from the same structure more commonly express Na currents with
distinct properties (Afshari et al. 2004; Aman and Raman 2007; Martina and Jonas 1997; but see also Huguenard et al. 1988).

Neurons that can sustain firing rates >50–60 Hz express ionic currents that are tuned to generate brief action potentials with short refractory periods (reviewed in Bean 2007). This tuning is often associated with the expression of Kv3-type K currents, but it is evident that some properties of voltage-gated Na currents are tuned to promote fast firing as well. These include rapid recovery from inactivated states as well as resistance to entry into slow inactivated states (Martina and Jonas 1997) and the expression of transient Na currents with resurgent kinetics, reflecting a mechanism of protection from fast inactivation during repetitive firing (Afshari et al. 2004; Do and Bean 2003; Mercer et al. 2007; Raman and Bean 1997; Raman et al. 2000). In Na,1,6 null mice, where resurgent Na currents are reduced, the maximum firing rates of some previously fast firing neurons were diminished to <50 Hz (Khaliq et al. 2003; Mercer et al. 2007; Van Wart and Matthews 2006), demonstrating the importance of this mechanism in supporting fast firing.

In slice recordings, most MVN neurons can sustain firing rates >150 spikes/s during DC current injections (Bagnall et al. 2007), placing them among the fastest firing neurons. In support of the link between a neuron’s firing capabilities and its expression of resurgent Na current, MVN neurons express large resurgent Na currents (13–15% the size of the transient Na current). Na currents from MVN neurons are exceptional in their ability to avoid accumulation into inactivated states. Na currents from MVN neurons are even faster to recover from inactivation than fast firing neurons in the hippocampus (Martina and Jonas 1997) and cerebellum (Aman and Raman 2007) and are more resistant to entry into the slow inactivated state. Both of these properties could enhance Na channel availability beyond levels observed in other fast firing neurons.

Although MVN neurons can sustain firing rates of hundreds of Hz in response to depolarizing current injection, in vivo firing rates have rarely been observed to exceed 100–200 Hz (Beraneck and Cullen 2007). Why do MVN neurons express Na currents that are so resistant to inactivation if their potential firing ranges are rarely utilized in vivo? The baseline firing rate of MVN neurons in vivo is typically around 50–60 Hz and in some neurons can be as high as or higher than 100 Hz (Beraneck and Cullen 2007), suggesting that Na currents in MVN neurons operate with a high basal level of Na current inactivation. The amplitude and biophysical properties of Na currents in GABAergic and non-GABAergic MVN neurons.
currents in MVN neurons ensure that even though Na currents are partially inactivated, neurons will be able to rapidly and reliably generate action potentials with the speed and precision required for the fast information processing required by the vestibular system.

Previous studies characterizing the firing properties of MVN neurons have distinguished MVN neurons based mostly on spike shape as type A or type B, which represent opposite ends of a continuous spectrum (Straka et al. 2005). TTX-sensitive plateau potentials were observed during firing in type B but not in type A MVN neurons, leading to the hypothesis that persistent Na currents are larger in type B compared with type A MVN neurons (Johnston et al. 1994; Serafin et al. 1991). Subsequent studies using rt-PCR have identified most type A neurons as GABAergic and type B neurons as either glutamatergic, glycinergic, or GABAergic (Bagnall et al. 2007; Taka-zawa et al. 2004). Although neurons in this study were distinguished by transmitter phenotype and not spike shape, most of the non-GABAergic neurons would be classified as type A and the GABAergic neurons would be classified as type B, based on their action potential widths and afterhyperpolarizations (Gittis and du Lac 2007). Although there is still a possibility that persistent Na currents are different between some type B and some type A neurons not sampled in this study, this study suggests that the absence of plateau potentials in type A neurons does not necessarily mean the absence of persistent Na currents. Plateau potentials present in type A neurons could be masked by a higher expression of K currents (Gittis and du Lac 2007), which has been observed in globus pallidus neurons (Lee and Tepper 2007).

Although Na current expression in GABAergic and non-GABAergic MVN neurons was quite similar, these cell types have different action potential waveforms and maximum firing rates (Bagnall et al. 2007; Gittis and du Lac 2007). One of the action potential parameters that most strongly distinguished these cell types was the presence of an afterdepolarization (ADP) in non-GABAergic but not GABAergic neurons (Bagnall et al. 2007). The finding that resurgent Na currents are large in both cell types suggests that resurgent current does not account for their differences in ADP. Differences in the outward currents have been described between these cell types and are likely to contribute to their different firing properties (Gittis and du Lac 2007). Additionally, subthreshold leak channels might also contribute to cell type differences between MVN neurons. A potassium leak current through TASK-3 channels facilitates sustained high firing rates in cerebellar granule cells (Brickley et al. 2007). Recently, a cyclic nucleotide-gated channel was identified in MVN neurons whose activation caused membrane depolarization and increased neuronal excitability, but this channel appeared to be expressed in most MVN neurons (Podda et al. 2008). An explanation of why non-GABAergic neurons are better able to utilize their Na currents to sustain higher maximum firing rates than GABAergic neurons will require a better understanding of how other currents influence Na channel availability during firing.

**FIG. 3.** Persistent Na currents are similar in both GABAergic and non-GABAergic neurons. A: a 1 μM TTX-sensitive persistent Na current (top) measured in a non-GABAergic neuron with a 115 mV/s ramp stimulus from −95 to +20 mV (bottom). B: current–voltage (I–V) curves of the average persistent Na currents measured in GABAergic ($n = 11$) and non-GABAergic ($n = 11$) neurons. The current reached similar peak amplitudes at −50 mV in both cell types. Error bars are SE.

**FIG. 4.** Resurgent Na currents are similar in both GABAergic and non-GABAergic neurons. A: resurgent Na currents from a non-GABAergic MVN neuron, observed at different potentials after a 10-ms voltage step to 0 mV (voltage protocol shown below). The transient current is truncated where indicated for clarity. B: I–V curves of the average resurgent Na currents measured in GABAergic ($n = 13$) and non-GABAergic neurons ($n = 14$). Error bars are SE. The resurgent Na currents were maximal at voltage steps to −35 mV in both cell types. Although non-GABAergic neurons tended to have more resurgent Na current, this difference was not significant ($P = 0.23$).
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