Sodium Currents in Neurons From the Rostroventrolateral Medulla of the Rat

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Rybak, Ilya A., Krzysztof Ptak, Natalia A. Shevtsova, and Donald R. McCrimmon. Sodium currents in neurons from the rostroventrolateral medulla of the rat. J Neurophysiol 90: 1635–1642, 2003. First published May 21, 2003; 10.1152/jn.00150.2003. Rapidly inactivating and persistent sodium currents have been characterized in acutely dissociated neurons from the area of rostroventrolateral medulla that included the pre-Bötzinger Complex. As demonstrated in many studies in vitro, this area can generate endogenous rhythmic bursting activity. Experiments were performed on neonate and young rats (P1-15). Neurons were investigated using the whole cell voltage-clamp technique. Standard activation and inactivation protocols were used to characterize the steady-state and kinetic properties of the rapidly inactivating sodium current. Slow depolarizing ramp protocols were used to characterize the noninactivating sodium current. The “window” component of the rapidly inactivating sodium current was calculated using mathematical modeling. The persistent sodium current was revealed by subtraction of the window current from the total noninactivating sodium current. Our results provide evidence of the presence of persistent sodium currents in neurons of the rat rostroventrolateral medulla and determine voltage-gated characteristics of activation and inactivation of rapidly inactivating and persistent sodium channels in these neurons.

INTRODUCTION

The persistent sodium current (I\textsubscript{NaP}) has been found important for intrinsic pacemaker activity in many types of neurons (e.g., see Alonso and Llinàs 1989; Bevan and Wilson 1999; Dickson et al. 1997; Llinàs et al. 1991; Pape and Driesang 1998; Pennartz et al. 1997; Takakusaki and Kitai 1997), yet it has been suggested that I\textsubscript{NaP} plays an essential role in the activation of pacemaker neurons in the pre-Bötzinger Complex (pBC), a region within the rostroventrolateral medulla (RVLM), in vitro (Butera et al. 1999; Del Negro et al. 2001; Smith et al. 2000). Del Negro et al. (2002) experimentally characterized I\textsubscript{NaP} in pBC neurons. This study, however, focused exclusively on a noninactivating component of sodium current. The authors did not characterize I\textsubscript{Na} and hence did not separate I\textsubscript{NaP} from the total noninactivating sodium current. The objective of our study was to experimentally characterize voltage-gated and kinetic properties of both I\textsubscript{Na} and I\textsubscript{NaP} in the area of RVLM including the pBC. A preliminary report of this work was published in abstract form (Shevtsova et al. 2002).

METHODS

Acutely dissociation procedure

Neurons were dissociated according to techniques described by McCrimmon et al. (2001). Briefly, newborn and young (1–15 days old) Sprague-Dawley rats were anesthetized using isoflurane and decapitated. Sagittal Vibratome (Series 1000, Vibratome, St Louis, MO) slices of the brain stem (300-μm-thick) were collected in Tyrode’s solution containing (in mM) 150 NaCl, 4 KCl, 2 CaCl\textsubscript{2}, 2 MgCl\textsubscript{2}, 10 HEPES, and 10 glucose, pH 7.4, 300 ± 5 mosM/l. The slices were incubated for 20 min at 33°C in oxygenated minimum essential medium (MEM; Life Technologies, Grand Island, NY) with (in mM) 10 HEPES, 5 l-cysteine and 0.5 EDTA, pH 7.2 to which 20 U/ml of papain (Worthington, Lakewood, NJ) was added. After enzymatic digestion, slices were washed three times in MEM-HEPES to which 1 mg/ml trypsin inhibitor and 1 mg/ml bovine serum albumin had been added.

Our intention was to study neurons from the region of RVLM that included the area of suggested location of the pBC. This area was defined in the sagittal slices by its characteristic distances from the fluorescent-labeled facial and compact/semicompact ambiguous nuclei. These cranial motoneurons were labeled by subcutaneous injection of Fluoro-Gold (50 mg/kg; Fluorochrome, Denver, CO) after birth (Ptak et al. 2001). While viewing the slices at low magnifications using an inverted microscope and epifluorescent ultraviolet illumination, a small region of the RVLM around the suggested location of the pBC was dissected from the slice and then dissociated mechanically using a series of fire-polished Pasteur pipettes. The cell suspension was centrifuged at 1,000 rpm for 10 min, then resuspended, plated onto 35-mm Petri dishes and placed on the stage of an inverted microscope.

Electrophysiological recordings

Borosilicate pipettes (WPI) were pulled, coated with Silicon elastomer (Sylgard, Dow Corning) and fire-polished. Electrode resistance was 1.5–4 MΩ when filled with internal solution containing (in mM) 130 N-methyl-d-glucamine, 10 EGTA, 20 HEPES, 10 CsCl, 2 MgCl\textsubscript{2}, 12 phosphocreatine, 2 Mg-ATP, 0.7 Na\textsubscript{2}GTP, and 0.1 leupeptin, pH 7.2 with CsOH/H\textsubscript{2}SO\textsubscript{4} (265 ± 5 mosM/l). The rapidly inactivating sodium current was recorded using an external solution consisting of (in mM) 15 NaCl, 110 TEA-Cl, 10 HEPES, 10 CsCl, 1 MgCl\textsubscript{2}, 2 BaCl\textsubscript{2}, and 0.3 CdCl\textsubscript{2} buffered to pH 7.4 with CsOH (300 ± 5 mosM/l). For measurement of persistent sodium current, the external...
solution contained (in mM) 115 NaCl, 45 TEA-Cl, 10 CsCl, 10 HEPES, 1 MgCl₂, 2 BaCl₂, and 0.3 CdCl₂, buffered to pH 7.4 with CsOH (300 ± 5 mM). To avoid enhancing effect of low extracellular [Ca²⁺] on the persistent sodium current (Su et al. 2001), Ca²⁺ was replaced by the same concentration of Ba²⁺ (Magistretti and Alonso 1999). Leak current was revealed by blocking sodium currents with 300 nM TTX and then subtracted. All reagents were obtained from Sigma (St. Louis, MO).

Data acquisition and analysis

Voltage-clamp recordings were made at room temperature (21–22°C) with an Axopatch 200A amplifier (Axon Instruments). After gigaseal formation and cell rupture, series resistance was compensated (80–90%) and monitored periodically. Data were acquired with PCLAMP-6 software, CLAMPEX and CLAMPFIT (Axon Instrument), and analyzed with IgorPro (WaveMetrics).

To characterize steady-state properties of \( I_{\text{NaP}} \), the standard activation and inactivation protocols were used (Hille 2001). To investigate the steady-state activation, currents were elicited by step depolarization from a holding potential of −80 mV to the potentials between −65 and +20 mV (using 5-mV steps). The conductance values for \( I_{\text{NaP}} \) were calculated by applying the extended Ohm’s law in the form: 

\[
g_{\text{NaP}} = I_{\text{NaP}} / (V - E_{\text{Na}}),
\]

where \( E_{\text{Na}} \) is the reversal (Nernst) Na⁺ potential calculated for the solution used in this protocol, and \( V \) is the step potential. Data were normalized and fit to the third-order Boltzmann function 

\[
g_{\text{NaP nominated}} = \text{mNaP} \times [1 + \exp(-(V - V_{1/2}\text{NaP})/k_{\text{NaP}})]^{-1},
\]

where \( \text{mNaP} \) is the steady state of activation variable, \( V_{1/2}\text{NaP} \) is the half activation voltage, and \( k_{\text{NaP}} \) is the slope factor.

To investigate the steady-state inactivation, currents elicited by a step depolarization to 20 mV from holding potentials between −115 and −20 mV (100-ms prepulse; 5-mV step). The steady-state inactivation curve was estimated by way of normalizing peak current, plotting the normalized currents versus potentials, and fitting the resultant curve to the first-order Boltzmann function 

\[
imax_\text{NaP}(t) = h_{\text{NaP max}} \times [1 + \exp(-(t - t_{\text{NaP max}})/k_{\text{NaP max}})]^{-1},
\]

where \( h_{\text{NaP max}} \) is the steady state of inactivation variable, \( t_{\text{NaP max}} \) is the half inactivation voltage, and \( k_{\text{NaP max}} \) is the slope factor.

The activation time constant, \( \tau_{\text{NaP max}}(V) \), was calculated from the time-to-peak \( t_r(V) \) data in each voltage step \( V \) during the activation protocol, fitting to first-order exponentials in the form: 

\[
t_r(V) = \tau_{\text{NaP max}}(V) \times [1 + \exp(-t_{\text{NaP max}}/k_{\text{NaP max}})],
\]

where \( \tau_{\text{NaP max}}(V) \) is the maximal value of activation time constant at \( V = V_{1/2}\text{NaP} \).

The inactivation time constant, \( \tau_{\text{NaP}}(V) \), was evaluated from the data on current decay for each voltage step during the activation protocol by fitting to first-order exponentials in the form: 

\[
t_r(V) = \tau_{\text{NaP}}(V) \times [1 + \exp(-t_{\text{NaP}}/k_{\text{NaP}})],
\]

where \( \tau_{\text{NaP}}(V) \) is the current value close to \( I_{\text{NaP}}(V) \) at the moment \( t_r \), and \( \tau_{\text{NaP}}(V) \) is the minimal (steady state) value of the decayed current. Similar to the activation time constant, the voltage dependence of inactivation time constant was fit to the function 

\[
\tau_{\text{NaP}}(V) = \tau_{\text{NaP max}}(V) \times [1 + \exp(-V - V_{1/2}\text{NaP})/k_{\text{NaP max}})]^{-1},
\]

where \( \tau_{\text{NaP max}}(V) \) is the half inactivation voltage, \( k_{\text{NaP max}} \) is the inactivation time constant slope factor, and \( \tau_{\text{NaP max}}(V) \) is the maximal value of inactivation time constant at \( V = V_{1/2}\text{NaP} \).

The experimentally characterized parameters for activation and inactivation of \( I_{\text{NaP}} \) \( V_{1/2}\text{NaP} \), \( \text{mNaP} \), \( \tau_{\text{NaP max}} \), \( k_{\text{NaP max}} \), \( V_{1/2}\text{NaP} \), \( \text{mNaP} \), \( \tau_{\text{NaP max}} \), \( k_{\text{NaP max}} \) were then incorporated in a computer model (see following text), which was used for simulation of the experimental activation protocol. The maximal conductance \( g_{\text{NaP}} \) was found in computer simulation to match the value of maximal sodium current (\( I_{\text{NaP max}} \)) in the model to that in the experimental protocol.

To experimentally characterize biophysical properties and voltage-gated characteristics of \( I_{\text{NaP}} \), a slow depolarizing ramp increase of voltage under voltage-clamp conditions is usually applied (e.g., see Magistretti and Alonso 1999; Parri and Crunelli 1998; Taddei and Bean 2002). It is considered that the rapidly inactivating component of sodium current (\( I_{\text{Nat}} \)) is eliminated under these experimental conditions. However, the problem persists because \( I_{\text{Nat}} \) has a nonactivating or “window” component resulting from the overlap of its voltage-dependent activation and inactivation characteristics (Baker and Bostock 1997; French et al. 1990; Magistretti and Alonso 1999). The window component (\( I_{\text{NatW}} \)) is comparable with the \( I_{\text{NaP}} \) in both the peak magnitude and activation threshold. Similar to the \( I_{\text{NaP}} \), the \( I_{\text{NatW}} \) persists when voltage is slowly increasing. Therefore, the voltage-gated characteristics of \( I_{\text{NaP}} \) if obtained without accounting the window current, are likely to characterize a mixture of persistent and window currents. Hence, to characterize a “pure” \( I_{\text{NaP}} \) it is necessary to study both \( I_{\text{Nat}} \) and \( I_{\text{NaP}} \) in the same neuron and to find a method allowing for separation of the \( I_{\text{Nat}} \) component from the total noninactivating sodium current. In the present study, we have made an attempt to characterize both fast and persistent sodium currents using a combination of experimental studies and computer modeling.

A slow depolarizing voltage ramp protocol was used to promote inactivation of the rapidly inactivating component of sodium current. The voltage was increased from −80 to 20 mV (at ramps of 100 and 75 mV/s). The evoked current (\( I_{\text{NaP tot}} \)) was considered to consist of the persistent sodium and window currents \( (I_{\text{NaP tot}} = I_{\text{Nat}} + I_{\text{NaP}}) \). The computer model was used to simulate the slow ramp protocol and find the voltage dependence of the window current \( I_{\text{NatW}} \). The \( I_{\text{NatW}} \) was obtained by subtracting the simulated window current from the experimentally measured total current \( (I_{\text{NaP tot}} = I_{\text{Nat tot}} - I_{\text{NatW}}) \). The conductance value for persistent sodium current was calculated from \( I_{\text{Nat tot}} \) by applying the extended Ohm’s law in the form: 

\[
g_{\text{Nat tot}} = I_{\text{Nat tot}} / (V - E_{\text{Na}}),
\]

where \( E_{\text{Na}} \) is the reversal Na⁺ potential calculated for the solution used in this protocol. Data were normalized and fit to the first-order Boltzmann function 

\[
g_{\text{Nat tot nominated}} = m_{\text{Nat tot}} \times [1 + \exp(-V - V_{1/2}\text{Nat tot})/k_{\text{Nat tot max}})]^{-1},
\]

where \( m_{\text{Nat tot}} \) is the steady state of activation variable, \( V_{1/2}\text{Nat tot} \) is the half activation voltage, \( k_{\text{Nat tot max}} \) is the slope factor, and \( g_{\text{Nat tot nominated}} \) is the maximal conductance of the persistent sodium current.

All average values were expressed as mean ± SD.

Mathematical and computer modeling

The \( I_{\text{NatW}} \) was described according to the classical Hodgkin-Huxley formalism

\[
I_{\text{NatW}} = g_{\text{NatW}} \times (V - E_{\text{Na}}),
\]

where the steady-state activation and inactivation were described as

\[
\tau_{\text{NatW}}(V) = \frac{d}{dt} m_{\text{NatW}}(V) = m_{\text{NatW}}(V) - m_{\text{NatW}},
\]

\[
\tau_{\text{NatW}}(V) = \frac{d}{dt} h_{\text{NatW}}(V) = h_{\text{NatW}}(V) - h_{\text{NatW}},
\]

and the voltage-dependent time constants for activation and inactivation were

\[
m_{\text{NatW}}(V) = [1 + \exp(-(V - V_{1/2}\text{NatW})/k_{\text{Nat tot max}})]^{-1},
\]

\[
h_{\text{NatW}}(V) = [1 + \exp(-(V - V_{1/2}\text{NatW})/k_{\text{Nat tot max}})]^{-1}.
\]
\[
\tau_{\text{Na}^+}(V) = \frac{\tau_{\text{Na}^+}}{\cosh(V - V_{1/2\text{Na}^+})k_{\text{Na}^+}} \\
\tau_{m\text{Na}^+}(V) = \frac{\tau_{m\text{Na}^+}}{\cosh(V - V_{1/2m\text{Na}^+})k_{m\text{Na}^+}}
\]

The \( I_{\text{Na}^+} \) had no inactivation and was described as follows

\[
I_{\text{Na}^+} = g_{\text{Na}^+} \cdot (V - E_{\text{Na}^+}) \\
g_{\text{Na}^+} = g_{\text{Na}^+} \cdot m_{\text{Na}^+}(V, t)
\]

\[
\tau_{\text{Na}^+}(V) \cdot \frac{dm_{\text{Na}^+}}{dt} = m_{\text{Na}^+}(V) - m_{\text{Na}^+} \\
m_{\text{Na}^+}(V) = \left\{ 1 + \exp[-(V - V_{1/2\text{Na}^+})k_{m\text{Na}^+}] \right\}^{-1} \\
\tau_{m\text{Na}^+}(V) = \frac{\tau_{m\text{Na}^+}}{\cosh(V - V_{1/2m\text{Na}^+})k_{m\text{Na}^+}}
\]

All simulations were performed on a Pentium IV computer, 1.7 GHz/512 MB (DELL) with a Windows 2000 operating system using MATLAB 6.0 (MathWorks) ode15s routine for solution of differential equations.

**RESULTS**

**Characterization of the rapidly inactivating sodium current**

Figure 1, A and B illustrates sodium currents in a representative neuron evoked by standard activation and inactivation protocols respectively. Steady-state plots (see Fig. 1C) were constructed for this neuron following the subtraction of the TTX-insensitive leak current. Activation data were re-calculated for normalized conductance and fit to the third-order Boltzmann function \( m_{\text{Na}^+} \). Where \( m_{\text{Na}^+} \) was defined by Eq. 3 (see METHODS). The first-order Boltzmann function \( h_{\text{Na}^+} \) (see Eq. 3 in METHODS) was used to fit the inactivation data (Fig. 1C).

Nine dissociated neurons were characterized. The average half activation voltage \( V_{1/2m\text{Na}^+} = -43.8 \pm 2.3 \text{ mV} \) and the slope factor \( k_{m\text{Na}^+} = 6.0 \pm 0.8 \text{ mV} \). The average half inactivation voltage \( V_{1/2h\text{Na}^+} = -67.5 \pm 3.6 \text{ mV} \) and the slope factor \( k_{h\text{Na}^+} = 10.8 \pm 2.4 \text{ mV} \). Figure 2A shows steady-state activation (fit to the 3rd-order Boltzmann function) and inactivation (fit to the 1st-order Boltzmann function) curves for nine neurons studied.

To characterize voltage dependence of time constants for
fast sodium activation and inactivation, we used the suggestion that these time constants reach their maximal values at the voltages of half activation and half inactivation, respectively. The obtained experimental data were fit to the functions $E = \frac{1}{2}m_{\text{Naf}}$ and $k_{\text{Naf}}$ for 9 neurons studied, where $m_{\text{Naf}}$ and $k_{\text{Naf}}$ are the voltage-dependent window current for each tested neuron. For example, for the representative neuron in Fig. 1, A–D, the following parameter values were used in simulation: $V_{1/2m_{\text{Naf}}} = -45.6\,\text{mV}$; $k_{m_{\text{Naf}}} = 6.9\,\text{mV}$; $V_{1/2k_{\text{Naf}}} = 1.0\,\text{mV}$; $k_{m_{\text{Naf}}} = 12.0\,\text{mV}$; $V_{1/2k_{\text{Naf}}} = -68.4\,\text{mV}$; $k_{\text{Naf}} = 10.1\,\text{mV}$; $\tau_{m_{\text{Naf}}} = 35.2\,\text{ms}$; $k_{m_{\text{Naf}}} = 12.7\,\text{mV}$; $E_{\text{Naf}} = 40\,\text{mV}$. The initial condition for voltage was set to $V(0) = -80\,\text{mV}$. Voltage steps were applied as follows

$$V(t, n) = -80 + 5 \cdot n \cdot \text{Sgn}(t) \quad n = 3, \ldots, 20$$

where $\text{Sgn}(x) = \{1, \text{if } x \geq 0; \ 0, \text{otherwise}\}$.

The value of $\tilde{g}_{\text{Naf}}$ was found in computer simulation to match the value of maximal sodium current ($I_{\text{max}}$) obtained in the corresponding experimental protocol at the voltage step producing maximal current amplitude. Figure 1E shows the results of computer simulation of the standard activation protocol for the representative neuron shown in Fig. 1, A–D. For this neuron, the maximal sodium current in the model (at the voltage step from $-80$ to $-25\,\text{mV}$) fit the experimentally defined maximal current of $2,255\,\text{pA}$ (revealed at the same voltage step) at $\tilde{g}_{\text{Naf}} = 73\,\text{nS}$ (see Fig. 1, E and F). In nine neurons studied, the maximal conductance for the rapidly inactivating sodium current (defined by the above method) was in the range of $\tilde{g}_{\text{Naf}} = 34 - 170\,\text{nS}$.

Characterization of the persistent sodium current

All of the neurons tested exhibited a non-inactivating, TTX-sensitive sodium current that was evoked by the slow (100 and 75 mV/s) ramp increase of membrane voltage from $-80$ to $20\,\text{mV}$. Figure 3 shows a noninactivating sodium current (after subtracting the TTX-insensitive component) in the representative neuron (same as in Fig. 1, A–D) obtained during the 75 mV/s ramp protocol. This current was activated at about $-60\,\text{mV}$ and reached a peak near $-40\,\text{mV}$ (Fig. 3). This total nonactivating current was presumed to consist of the persistent sodium current and the window component of the rapidly inactivating sodium currents: $I_{\text{Naf tot}} = I_{\text{Naf}} + I_{\text{Naf W}}$.

The voltage-dependent window current for each tested neuron was obtained using computer simulation. The model was used to simulate experimental voltage-ramp protocols. The value of maximal conductance for the rapidly inactivating sodium current was

$$g_{\text{Naf tot}} = \frac{I_{\text{Naf tot}}}{V_{\text{Naf tot}} - V_{\text{Naf W}}},$$

where $V_{\text{Naf tot}}$ is the voltage corresponding to the maximal sodium current amplitude.

...
corresponding experimental protocols \((/H1100280\text{mV})\). The voltage increased with the ramp used in the simulation of the voltage ramp protocol (75 mV/s), to the steady-state traces of these variables. This comparison shows that the transient component of the rapidly inactivating sodium current is almost eliminated, and the remaining window current is close to its steady state.

(fast) sodium current \((\hat{I}_{\text{NaP}})\) for each tested neuron was found in advance during simulation of the standard activation protocols. The initial condition for voltage was set to \(-80\text{ mV} [V(0) = -80\text{ mV}]\). The voltage increased with the ramp used in the corresponding experimental protocols \((v = 100\text{ mV/s} = 0.1\text{ mV/ms or } 75\text{ mV/s} = 0.075\text{ mV/ms})\). Figure 4 shows an example of this comparison for the representative neuron obtained in simulation of the 75-mV/s voltage ramp protocol.

The persistent sodium current \((I_{\text{NaP}})\) was calculated by subtracting the window current \((I_{\text{NaW}})\) obtained in simulation from the total sodium current \((I_{\text{NaTot}})\) obtained in the ramp voltage protocol.

\[
I_{\text{Na}} = I_{\text{NaTot}} - I_{\text{NaW}}
\]

Figure 5A shows an example of \(I_{\text{NaTot}}\) and \(I_{\text{NaW}}\) in the representative neuron. The conductance of the persistent sodium current was calculated from \(I_{\text{NaP}}\) data by applying Ohm’s law: \(g_{\text{NaP}} = I_{\text{NaP}}/(V - E_{\text{Na}})\). An example for the representative neuron is shown in Fig. 5C. The values of conductance were normalized and fit to the first-order Boltzmann function \(g_{\text{NaP}}/g_{\text{NaW}} = m_{\text{NaP}}/m_{\text{NaW}}\) (see Eq. 5 in Methods and an example in Fig. 5, D and E).

For nine neurons characterized in two voltage ramp protocols, the average half activation voltage for the persistent sodium current and the slope factor were, respectively: \(V_{1/2m_{\text{NaP}}} = -45.6 \pm 3.1\text{ mV} \) and \(k_{m_{\text{NaP}}} = 3.0 \pm 0.4\text{ mV} \) (for the 100-mV/s protocol) and \(V_{1/2m_{\text{NaP}}} = -48.6 \pm 3.5\text{ mV} \) and \(k_{m_{\text{NaP}}} = 3.2 \pm 0.4\text{ mV} \) (for the 75-mV/s protocol). Figure 6, A and B, shows steady-state activation curves (fit to the Boltzmann function) for nine neurons studied in each experiment.
ramp protocol. Considering both voltage ramp protocols, the average half activation voltage for persistent sodium current in the characterized neurons \( V_{1/2mNaP} = -47.1 \pm 4.1 \) mV and the slope factor \( \kappa_{mNaP} = 3.1 \pm 0.4 \) mV (Fig. 6C). The maximal conductance of the persistent sodium channels in nine neurons studied was in the range of \( g_{NaP} = 0.5 - 5.5 \) nS.

To make a backward comparison between the values for \( I_{NaP} \) and \( I_{NaTot} \) obtained in simulation and those from experimental studies, we ran simulations of the corresponding ramp protocols using the parameters established for the rapidly inactivating and persistent sodium currents in each characterized neuron

\[
I_{Na} = g_{Na} \cdot m_{Na} \cdot (V - E_{Na})
\]

\[
I_{NaTot} = I_{Na} + I_{NaP} = g_{Na} \cdot m_{Na} \cdot h_{Na} \cdot (V - E_{Na}) + g_{NaP} \cdot m_{NaP} \cdot (V - E_{Na})
\]

Examples of matching the experimental and model curves for \( I_{NaP} \) and \( I_{NaTot} \) for the representative neuron are shown in Fig. 7, A and B.

As seen in Figs. 5D and 7, A and B, the theoretical curves for both the persistent sodium conductance and the current fit well \((R^2 > 0.95)\) to the corresponding experimental curves for the initial values of voltage \((V < -35 \) mV), where \( I_{NaP} \) is activated. They also fit well for the values of voltage beyond the window current \((V > -5 \) mV), where the window current is almost completely inactivated and the persistent sodium current is completely activated. This supports the correctness of our estimation of both the voltage-gated characteristics and the maximal conductance of the persistent sodium current. At the same time, there is a significant divergence between the curves within the voltage interval corresponding to inactivation of the window current (see Figs. 5D and 7, A and B). This divergence may slightly diminish the estimated parameters of channel kinetics. It could result from a number of reasons, such as overly simplistic formal description used for both rapidly inactivating and persistent sodium channels, differences in the inactivation of the rapidly inactivating sodium current between the standard and ramp experimental protocols, disregarding the maintained presence of the persistent sodium component during processing data from the standard inactivation protocol. The divergence between the experimental and theoretical curves for persistent sodium current in the range from \(-35\) to \(-5\) mV could also be connected with anomalous properties of \( I_{NaP} \) channel noise. Kay et al. (1998) studied steady-state noise characteristics of the persistent sodium conductance experimentally and found that variance of the persistent sodium current is relatively small for voltages less than \(-40\) mV, then dramatically rises in a range from \(-35\) to \(-25\) mV and monotonically decreases for voltages greater than \(-25\) mV. The maximal value of variance in their experiments was in a voltage range from \(-35\) to \(-5\) mV, which corresponds to the largest divergence between the experimental and theoretical curves for persistent sodium current in our study.

**Discussion**

Some limitations of the present study

The neurons characterized in this study were taken from animals of different ages (P1–P15), and hence possible development-dependent or age-dependent sodium channel expression could influence the variability of our results. Also our studies were performed on acutely dissociated neurons, which retained little of the original axons and dendrites. Therefore characteristics obtained in this study likely relate to somatic and proximal dendritic sodium channels.

In the present study, the maximal conductance of the rapidly inactivating sodium current was determined by matching the simulated and experimental curves corresponding to one step...
of the standard activation protocol. Specifically, we used the step that produced maximal current in both simulation and experiment. We did not compare the entire family of curves obtained in simulation with the entire family of experimental curves. A more precise method could be based on the comparison of partial or full families of curves. This idea was not implemented in the present study, but may be used in future investigations.

The present experimental study, including its modeling component, was performed using the assumption that $I_{\text{NaP}}$ and $I_{\text{Na}}$ result from the movement of sodium ions through distinct ionic channels. At the same time, it is currently unknown whether these channels are indeed separate or represent different states of uniform but kinetically more complicated sodium channels. This issue is a subject of intense debate (e.g., see Baker and Bostock 1997; Crill 1996; Parri and Crunelli 1998; Taddese and Bean 2002).

Rapidly inactivating sodium channels

This report represents the first attempt to characterize rapidly inactivating sodium currents in RVLM neurons. Analysis of the literature has shown that the steady-state characteristics of voltage-dependent activation and inactivation of $I_{\text{Na}}$ in the RVLM neurons are similar to these in neurons from other regions in the rat brain. Our data also demonstrate that rapidly inactivating sodium current in RVLM neurons has a relatively large window conductance that may produce a window component of the rapidly inactivating sodium current comparable with the persistent sodium current in the same neuron.

Persistent sodium channels

The presence of these channels in RVLM and pBC neurons has been previously reported (Del Negro et al. 2002; Koshiya et al. 2001; McCrimmon et al. 2001; Ptak et al. 2001). Koshiya et al. (2001) reported that the peak conductance of the persistent sodium channels in pBC neurons ranged from 2.4 to 4.5 nS. According to the experimental estimation of Del Negro et al. (2002), this conductance reaches a peak value of 4–5 nS. Our measurements (0.5–5.5 nS) are consistent with these data.

Comparative analysis shows that the half activation voltage for the persistent sodium current in neurons located in different brain areas is usually about or slightly more positive than −50 mV (see Table 1). Our data on the persistent sodium half activation voltage (−47.1 ± 4.1 mV) are reasonably close to this general estimation. Consequently, we assume that the voltage-gated characteristics of the persistent sodium current in the RVLM are similar to those reported for neurons from other brain areas.

Del Negro et al. (2002) reported that the half activation voltage for $I_{\text{NaP}}$ in pBC neurons was about −40 mV, which significantly differs from our data for RVLM neurons and data on $I_{\text{NaP}}$ in neurons from other brain areas (see Table 1). In contrast to our study, Del Negro et al. (2002) characterized sodium current without considering the contribution of the window component of rapidly inactivating sodium current that, as shown herein, is significant in RVLM neurons. This, however, cannot explain the positive shift in the half activation voltage in their data relative to our results. Also Del Negro et al. (2002) conducted functional identification of neurons before the investigation of $I_{\text{NaP}}$ characteristics. They, therefore, could specifically characterize pacemaker neurons in the pBC and separate them from nonpacemaker neurons. We did not perform functional identification of characterized neurons, and hence our data relate to functionally nonidentified RVLM neurons. However, Del Negro et al. (2002) reported a lack of significant difference in voltage-gated characteristics of the $I_{\text{NaP}}$ between pacemaker and nonpacemaker neurons. Therefore it is unlikely, that the difference in our data occurred because of the lack of functional identification in our studies. The other difference between the two studies was that we characterized the persistent sodium current in acutely dissociated neurons (and mostly in somatic currents), whereas Del Negro et al. (2002) characterized neurons in slices.

In summary, results of the present study provide additional evidence of the presence of the persistent sodium current in RVLM neurons. The voltage-dependent characteristics of activation and inactivation of $I_{\text{NaP}}$ and $I_{\text{Na}}$ drawn from our experiments may provide a necessary basis for computational modeling and analysis of RVLM neurons.

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