Contribution of Persistent Sodium Currents to Spike-Frequency Adaptation in Rat Hypoglossal Motoneurons

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Zeng, Jinsong, Randall K. Powers, Gregory Newkirk, Marc Yonkers, and Marc D. Binder. Contribution of persistent sodium currents to spike-frequency adaptation in rat hypoglossal motoneurons. J Neurophysiol 93: 1035–1041, 2005. First published September 8, 2004; doi:10.1152/jn.00831.2004. In response to constant current inputs, the firing rates of motoneurons typically show a continuous decline over time. The biophysical mechanisms underlying this process, called spike-frequency adaptation, are not well understood. Spike-frequency adaptation normally exhibits a rapid initial phase, followed by a slow, later phase that continues throughout the duration of firing. One possible mechanism mediating the later phase might be a reduction in the persistent sodium current ($I_{NaP}$) that has been shown to diminish the capacity of cortical pyramidal neurons and spinal motoneurons to sustain repetitive firing. In this study, we used the anticonvulsant phenytoin to reduce the $I_{NaP}$ of juvenile rat hypoglossal motoneurons recorded in brain stem slices, and we examined the consequences of a reduction in $I_{NaP}$ on the magnitude and time course of spike-frequency adaptation. Adding phenytoin to the bathing solution ($\approx 50 \mu M$) generally produced a marked reduction in the persistent inward currents (PICs) recorded at the soma in response to slow, voltage-clamp triangular ramp commands ($-70$ to $0$ mV and back). However, the same concentrations of phenytoin appeared to have no significant effect on spike-frequency adaptation even though the phenytoin often augmented the reduction in action potential amplitude that occurs during repetitive firing. The surprising finding that the reduction of a source of sustained inward current had no appreciable effect on the pattern of spike generation suggests that several types of membrane channels must act cooperatively to insure that these motoneurons can generate the sustained repetitive firing required for long-lasting motor behaviors.

INTRODUCTION

The discharge rate of motoneurons is a function of both the amplitude and time course of synaptic inputs delivered to the spike-generating conductances in the soma and axon. In response to a step of input current, the discharge rate typically declines as a function of time since current onset, a process known as spike-frequency adaptation (see review in Powers and Binder 2001). Spike-frequency adaptation has a rapid initial phase (Kernell 1965; Sawczuk et al. 1995b, 1997), followed by a slower, later phase that continues throughout the duration of firing (late adaptation; Granit et al. 1963; Kernell 1965; Kernell and Monster 1982; Sawczuk et al. 1995a,b; Spielmann et al. 1993). The short interspike intervals that typically occur at the onset of an excitatory input to a motoneuron increases the speed of force development in its innervated muscle fibers (Baldissera et al. 1987). The later slow decline in firing rate can allow motor units to maintain a steady force by matching increases in twitch contraction times with a decrease in activation rate (Bigland-Ritchie et al. 1983; Sawczuk et al. 1995a), although it may eventually lead to a significant decline in force output (Kernell and Monster 1982).

Despite the clear functional significance of spike-frequency adaptation, its underlying biophysical mechanisms are not well understood. Quantitative analysis of the time course of spike-frequency adaptation in rat hypoglossal motoneurons (Sawczuk et al. 1995b) reveals that most cells respond to a long step of injected current with three, temporally distinct phases of adaptation: initial, early, and late. The initial phase consists of a rapid drop in frequency that is a linear function of time. This phase is followed by a more gradual decline that is generally fit by the sum of two exponential functions. The early process has a time constant on the order of $250 \text{ ms}$ and is followed by a slower process that has a time constant on the order of $10–20 \text{ s}$. It is still not clear whether or not there are different biophysical mechanisms underlying the different phases of adaptation (see review in Powers and Binder 2001; Powers et al. 1999).

Much of the previous research on the initial and early phases of adaptation has examined the role of the medium-duration afterhyperpolarization (mAHP) that follows each action potential. The mAHP results from a calcium-sensitive potassium conductance ($G_{KCa}$) activated by calcium entry during the action potential. The accumulation of calcium across successive spikes will lead to summation of the mAHP conductance and a decrease in firing rate (see review in Powers and Binder 2001). Earlier work indicated that mAHP summation plays a prominent role in initial adaptation (Baldissera and Gustafsson 1974; Baldissera et al. 1978; Kernell 1968). However, more recent experimental and simulation results suggest that mAHP summation is probably not responsible for the later phases of adaptation (Powers et al. 1999). Early and late adaptation are still present when the mAHP is eliminated by replacing external calcium with manganese (Powers et al. 1999; Sawczuk et al. 1997).

A variety of other mechanisms could contribute to the later phases of adaptation. A slowly activating $G_{KCa}$ conductance that is pharmacologically distinct from that underlying the mAHP has been described in mammalian neocortical and hippocampal neurons (Madison and Nicoll 1984; Schwindt et al. 1988; Storm 1990). This $G_{KCa}$ conductance contributes both to a long-lasting AHP (sAHP) following repetitive discharge and to slow spike-frequency adaptation in these cells. A sodium-activated calcium-conductance may also contribute to the
sAHP in neocortical neurons (Schwindt et al. 1989). However, there is no convincing evidence for functionally important sAHPs in mature motoneurons (Powers and Binder 2001). Furthermore, there is little evidence that other slowly developing outward currents contribute to spike-frequency adaptation in motoneurons (see Discussion).

A slow decline in inward currents could also contribute to adaptation. Spike-frequency adaptation in both cat and rat motoneurons is associated with changes in the shape of action potentials. During long periods of repetitive discharge, there is a progressive decrease in spike height, an increase in spike duration, and a decrease in both the maximum rate of depolarization and rate of repolarization of the action potential (Musick 1999; Sawczuk 1993). In addition, motoneurons have both persistent sodium and persistent calcium currents activated in the voltage range between resting potential and spike threshold (Carlin et al. 2000; Hsiao et al. 1998; Lee and Heckman 1998a, 2000; Powers and Binder 2003). Progressive inactivation of these inward currents would be expected to result in a decreased firing rate. Whereas the low-threshold calcium current often shows facilitation rather than inactivation (Bennett et al. 1998; Svirskis and Hounsfield 1997), the persistent sodium current has been shown to undergo slow inactivation in neocortical neurons (Fleidervish et al. 1996) and spinal motoneurons (Lee and Heckman 2001).

The aim of this study was to consider the potential contribution of the slowly inactivating persistent sodium current ($I_{\text{NaP}}$) to spike-frequency adaptation in rat hypoglossal motoneurons. We used the anticonvulsant phenytoin to reduce the $I_{\text{NaP}}$ of juvenile rat hypoglossal motoneurons recorded in brain stem slices and examined the consequences for the magnitude and the time course of spike-frequency adaptation (cf. Lampa et al. 1998). Phenytoin appeared to be the agent of choice because it has been shown to block Na channels in a use-dependent manner: when studied with trains of action potentials, the blockade is more pronounced late in the train (reviewed in Kuo and Bean 1994). As expected, adding phenytoin ($\geq 50 \mu M$) to the bathing solution generally produced a marked reduction in the persistent inwards currents (PICs) recorded at the soma in response to slow, voltage-clamp triangular ramp commands ($–70$ to $0 \text{ mV}$ and back). However, phenytoin appeared to have no significant effect on spike-frequency adaptation even though the drug often augmented the reduction in action potential amplitude that occurs during repetitive firing. The surprising finding that the reduction of a source of sustained inward current had no appreciable effect on the pattern of spike generation suggests that several types of membrane channels must act cooperatively to insure that these motoneurons can generate the sustained repetitive firing required for long-lasting motor behaviors (Powers and Binder 2001; Powers et al. 1999).

**Solutions and chemicals**

To minimize neural activity during the initial preparation of the slices, two different modified ACSF solutions were used. For slices obtained from younger animals (12–16 days), we used a low-Ca²⁺, high-Mg²⁺ solution ([low Ca-ACSF (in mM): 132 NaCl, 3 KCl, 1·25 NaH₂PO₄, 26 NaHCO₃, 5 MgCl₂, 1 CaCl₂, and 10 D-glucose], whereas in older animals, we used a sucrose-based solution (S-ACSF: same as low Ca-ACSF, except 220 mM sucrose was substituted for NaCl, and concentrations of MgCl₂ and CaCl₂ were both 2 mM). Kynurenic acid (1 mM) and sodium lactate (4 mM) were also added to the initial incubation medium to improve cell viability. The standard ACSF was identical to that of the S-ACSF except that 132 mM NaCl was substituted for sucrose. The pH of the ACSF solutions ranged from 7.3 to 7.4, and their measured osmolalities ranged from 310 to 320 mOsm.

To study the entire ensemble of whole cell currents, we used a patch recording solution containing (mM) 146 KCH₃SO₄, 5 KCl, 2 MgCl₂, 2 EGTA, 10 MOPS, 2 Na₃ATP, and 0.2 Na₃GTP. KOH/HCl was added to bring the pH to 7.2. The osmolality of this solution was 310 mOsm. Patch solution aliquots were stored at $–20^\circ\text{C}$ until time of use.

To minimize the contribution of potassium currents, the pipette solution was composed of (mM) 100 CsCl, 20 TEACl, 5 MgCl₂, 2 BAPTA, 10 HEPES, 5 Na₃ATP, 0.5 Na₃GTP, and CsOH/HCl for 7.3 pH, and sucrose was added to bring the osmolality to 305 mOsm. To further isolate sodium currents, in some experiments, we also added 4 mM 4-aminopyridine (4-AP) and 10 mM tetraethylammonium chloride (TEACl–) to the bathing solution to block potassium channels and replaced CaCl₂ with MnCl₂ to eliminate calcium currents. Variations in NaCl content of the bathing solution were made to effect similar osmolalities when used with added K⁺ channel blockers. The NaCl concentration was 120 mM when combined with 4 mM 4-AP and 10 mM TEACl– to block potassium channels.

Phenytoin (Sigma Chemical, St. Louis, MO) was prepared as a 200-mL stock solution in DMSO. Aliquots of this stock solution were added to the bathing solution to obtain final phenytoin concentrations of $\leq 100 \mu M$.

**Whole cell patch recordings**

Whole cell recordings were obtained with patch electrodes placed on the somata of rat hypoglossal motoneurons under visual control using a Zeiss Axioskop equipped with Nomarski optics for differential interference contrast (DIC) and infrared video recording. The patch electrodes were glass pipettes with tip diameters of 1–2 μm and resistances of 2–4 MΩ when filled with the pipette solution. Electrical recordings were made with an Axon Instruments Multiclamp 700A amplifier and digitized at 10 kHz using an Instrutech A/D board connected to a Macintosh PowerPC. Data acquisition and voltage-clamp commands were controlled by custom software routines running in Igor (WaveMetrics).

Following the establishment of whole cell recording, the membrane potential was clamped at –70 mV. Whole cell currents were measured in response to slow (typically 14 or 28 mV/s), triangular voltage-clamp commands from –70 to 0 mV and back. In the illustrated current records (Fig. 1), the linear leak component has been subtracted either on-line by the amplifier’s circuitry or off-line based on scaling the responses to voltage changes within 10 mV of the holding potential. Following a change in the perfusate solution (i.e., control,
We used an Axon Instruments Axoclamp 2B amplifier and glass micropipettes filled with 3 M KCl (resistances of 30–80 MΩ). Intracellular recordings with sharp electrodes were obtained in hypoglossal motoneurons. A: leak-subtracted current-voltage plots derived from the responses to a series of voltage-clamp ramps from –70 to 0 mV at a rate of 14 mV/s with potassium channels blocked by CsCl, 4-aminopyridine (4-AP), and tetraethylammonium chloride (TEACl). PIC (black trace) was reduced by adding 100 μM phenytoin to the bath (red trace), particularly in the lower-voltage activated range (–55 to –35 mV), which is TTX-sensitive. Washing out the phenytoin (blue trace) resulted in a marked recovery, which was reversed by reintroducing phenytoin to the bath (green trace).

**Data analysis**

Analysis of spike-frequency adaptation as defined in Sawczuk et al. (1995b) was performed on every 30-s trial of repetitive discharge. The initial linear decline in discharge rate with respect to time was calculated as the difference between the initial firing rate, \( f_i \), and the transition firing rate, \( f_t \) (cf. Fig. 2). The extent of the initial adaptation was determined by fitting a line to an increasing number of interspike intervals until the \( r^2 \) value of the linear correlation fell below 0.75. The subsequent decline in firing rate was fit with a single exponential function as the difference between \( f_i \) and the average firing rate over the last second of discharge (\( f_b \)). The form of the single exponential fit was

\[
\text{Firing Rate} = f_i + K_0 \times \exp(-t/T_1)
\]

where \( K_0 \) is the magnitude and \( T_1 \) is the time constant of the later phase of adaptation. When this single exponential fit failed to account for the entire drop in firing rate, the time course of the later phases of adaptation (\( f_t \) to \( f_b \)) was fit with the sum of two exponential functions by “peeling away” the fit to the first exponential curve and fitting the remainder with a second exponential function (Sawczuk et al. 1995b; Spielmann et al. 1993). We also measured the height of all of the action potentials during each epoch of repetitive firing. Action potential height was measured from the threshold voltage (defined as the 1st point where the rate of change of membrane potential exceeded 10 mV/ms) to the peak amplitude.

**Effects of phenytoin on persistent inward currents**

We studied the effects of phenytoin on persistent inward currents (PICs) in 20 hypoglossal motoneurons recorded in brain stem slices taken from rats 12–22 days of age. As we have previously reported, in the presence of potassium channel blockers, rat hypoglossal motoneurons manifest large PICs that are activated at somatic membrane potentials around –60 to –50 mV (Powers and Binder 2003). The PICs are mediated by both Na\(^+\) and Ca\(^{2+}\) channels. Figure 1A shows the leak-subtracted current-voltage plots derived from the responses of a hypoglossal motoneuron to a series of voltage-clamp ramps from –70 to 0 mV at a rate 14 mV/s. The prominent PIC in this cell (\( >300 \) pA) was dramatically reduced by the addition of phenytoin (100 μM) into the bathing solution (red trace). In particular, the lower-voltage activated portion of the response...
presence of external calcium, the small persistent inward current (<125 pA; black trace) was presumably mediated by Na\(^+\) channels (Powers and Binder 2003). Again, adding 100 \(\mu\)M phenytoin to the bathing solution blocked nearly all of the persistent Na\(^+\) current (red trace). This figure also shows that phenytoin affects the transient Na\(^+\) current as evidenced by a reduction in the uncontrolled inward current transients at the onset of the voltage ramp.

**Effects of phenytoin of spike-frequency adaptation**

We studied the effects of phenytoin (20–100 \(\mu\)M) on repetitive firing and spike-frequency adaptation in a total of 31 rat hypoglossal motoneurons. However, our detailed analysis rests heavily on seven cells in which we acquired multiple 30-s epochs of repetitive firing under both control conditions \((n = 115\) trials) and after the application of 50 \(\mu\)M \((n = 70\) trials) and/or 100 \(\mu\)M phenytoin \((n = 43\) trials) to the bathing solution.

Figure 2 displays the typical pattern of spike-frequency adaptation observed in rat hypoglossal motoneurons in response to 30-s steps of injected current (Sawczuk et al. 1995b) and our principal finding that phenytoin appeared to have no significant effect on the magnitude or time course of spike-frequency adaptation. In Fig. 2A, there are two superimposed plots for the same cell in response to identical current steps of 1.4 nA: the black trace was obtained under control conditions and the red trace after 100 \(\mu\)M phenytoin was added to the bathing solution. The initial firing rates were identical in the two trials (170 imp/s), as were the firing rates at the end of the 30-s epochs (26 imp/s). Furthermore, the entire time courses of the later phases of the spike-frequency adaptation were virtually identical. Although phenytoin increased the rheobase current \([0.25 \pm 0.11 (SE) nA]\); Wilcoxon signed-rank test; \(z = -2.4, P < 0.02\) as previously reported for rat cortical neurons (Lampl et al. 1998), we found no consistent effect of phenytoin on the relationship between injected current and initial firing rate.

The graphs in Fig. 3 displays the relationship between the total amount of spike-frequency adaptation (i.e., \(f_i - f_0\)) and the initial firing rate (Fig. 3A), the relationship between the magnitude of the initial phase of spike-frequency adaptation and the initial firing rate (Fig. 3B), and the relationship between the later phases of frequency adaptation (i.e., early + late adaptation) and the firing rate at the end of the initial phase of adaptation (Fig. 3C) for a total of 228 epochs (30 s) of repetitive firing. The open circles in the graphs represent the control trials, filled circles represent the trials recorded in 50 \(\mu\)M phenytoin, and the asterisks show the data for 100 \(\mu\)M phenytoin. As we have previously reported (Sawczuk et al. 1995a,b, 1997), the magnitudes of the initial adaptation and the total adaptation are strongly correlated with the initial spike frequency \((r = 0.98, P < 0.001\) and \(r = 0.99, P < 0.001,\) respectively), and the magnitude of the early and late phases of adaptation are strongly correlated with the firing rate at the end of the initial adaptation \((r = 0.82, P < 0.001). None of these relationships showed significant differences between the control and phenytoin trials.

Thus, although treatment with 50 or 100 \(\mu\)M phenytoin reduces the persistent sodium current in rat hypoglossal mo-
trials in 50 phases of adaptation (i.e., early motoneurons. B
the action potential in rat hypoglossal motoneurons (Musick
maximum rate of depolarization and rate of repolarization of
height, an increase in spike duration, and a decrease in both the
repetitive discharge, there is a progressive decrease in spike
repetitive firing
Effects of phenytoin on action potential amplitude during
spike-frequency adaptation during 30-s repetitive firing trials. A: total spike-
frequency adaptation (i.e., $f_i - f_f$) vs. initial firing rate for 7 rat hypoglossal
motoneurons. B: initial adaptation vs. initial firing rate for same cells. C: later
phases of adaptation (i.e., early + late) vs. transition firing rate ($f_i$, firing rate
after initial phase of adaptation) for same cells. ○, control trials ($n = 115$); ●,
trials in 50 μM phenytoin ($n = 70$); *, trials in 100 μM phenytoin ($n = 43$).
toneurons (cf. Fig. 1), it appears to have no significant effect on
spike-frequency adaptation.

Effects of phenytoin on action potential amplitude during repetitive firing

We had previously reported that, during long periods of
repetitive discharge, there is a progressive decrease in spike
height, an increase in spike duration, and a decrease in both the
maximum rate of depolarization and rate of repolarization of
the action potential in rat hypoglossal motoneurons (Musick
1999; Sawczuk 1993). The decrease in spike height reflects
both an increase in the voltage threshold for spike initiation and
a decrease in the peak spike voltage (Powers et al. 1999).

Figure 2B displays the amplitudes of the action potentials
measured during the same repetitive firing trials depicted in
part of the figure. During the initial and early phases of
spike-frequency adaptation, the decline in spike height is
similar in the control (black) and phenytoin (red) trials. How-
ever, during the late phase of adaptation, there is a greater
reduction in spike height in the phenytoin trial. In Fig. 2C, we
display (from left to right) the first action potential from each
trial, followed by averages of 10 spikes occurring at the end of
1, 5, and 30 s after current onset during both the control (black)
and phenytoin (red) trials. By the end of the trial, the action
spike height in the phenytoin trial was only 43 mV, whereas
that in control was 50 mV.

We quantified the change in spike height during the later
phases of adaptation in two ways. First, we plotted the rela-
tionship between spike height and firing rate from the end of
the initial adaptation ($f_i$) to the end of the entire trial ($f_f$) and
calculated the best linear fit to each of these spike height versus
firing rate plots. The slope of this relationship was generally
higher in phenytoin, indicating a steeper decline in spike height
as a function of firing rate. For the same seven motoneurons we
used for the analysis of adaptation, the average slopes of the
spike height versus firing rate relations were $0.98 \pm 0.64$
$mV/imp/s$ under control conditions ($n = 115$ trials), $1.16 \pm$
$0.46 mV/imp/s$ after the application of 50 μM phenytoin ($n =
70$ trials), and $1.38 \pm 0.78 mV/imp/s$ after the application of
100 μM phenytoin ($n = 43$ trials) to the bathing solution. The
effect of phenytoin treatment was significant (ANOVA, $F =$
$6.71, P < 0.01$), and the mean slope after applying 100 μM
phenytoin was significantly higher than the mean control slope
($P < 0.001$).

We also quantified the change in spike height by simply
taking the difference in the spike height measured at the end of
initial adaptation and the average spike height for the last 10
spikes. Figure 4 displays the change in spike height between
$f_i$ and $f_f$ as a function of $f_i$. There was a strong correlation
between the change in spike height during the later phases of spike-
frequency adaptation and firing rate for the combined data ($r =$
$0.64; P < 0.001$), as well as for the control trials (○: $r = 0.58;
DISCUSSION

We had several reasons to expect that applying the anticonvulsant agent, phenytoin to rat hypoglossal motoneurons would affect the later phases of spike-frequency adaptation. It had previously been shown that phenytoin (≥50 μM) reduces the persistent sodium current in rat cortical neurons and enhances “slow adaptation” (Lampl et al. 1998). In addition, it has been shown in cat spinal motoneurons that modest reductions in the persistent sodium current results in profound changes in the slope of the current-frequency relation and can lead to a failure of regular repetitive firing (Lee and Heckman 2001). Furthermore, based simply on the monotonic current-frequency relation of motoneurons, one would surmise that any agent or regimen that decreases a source of inward current should lead to a decrease in firing rate (i.e., an increase in the later phases of spike-frequency adaptation). Similarly, one would surmise that decreasing a source of depolarizing current as we have done in our previous studies (Powers et al. 1999; Sawczuk et al. 1995b, 1997) are a possible reason for failed adaptation to repetitive firing. This difficulty raises the question of why mechanisms should be in place to insure that the pattern of spike-frequency adaptation is so resistant to disruption in motoneurons.

As discussed in the Introduction, the change in motoneuron firing rate during a sustained contraction has important consequences for force generation: an initial high firing rate is needed for the rapid development of force, whereas the subsequent decline in firing rate is matched to the change in muscle properties to ensure steady force production. Many of the channels activated during repetitive discharge are under neuromodulatory control (reviewed in Powers and Binder 2001). The contribution of multiple channels to spike-frequency adaptation may ensure that neuromodulatory regulation of motoneuron excitability (e.g., by altering membrane resistance, resting membrane potential, or the delivery of synaptic current to the soma) does not disrupt the normal time course of adaptation. It is also possible that the remarkably stable discharge patterns that we have observed here and in our prior studies (Powers et al. 1999; Sawczuk et al. 1995b, 1997) are a specialized feature of hypoglossal motoneurons to accommodate their essential contributions to respiration and feeding.

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