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

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ABSTRACT

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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 ($\geq$ 50 μM) 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 mV 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 (rev. 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; Spielmann et al. 1993; Sawczuk et al. 1995a,b). 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).

In spite of 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 ms, and is followed by a slower process
that has a time constant on the order of 10 - 20 s. It is still not clear whether or not there are different biophysical mechanisms underlying the different phases of adaptation (rev. in Powers et al. 1999; Powers and Binder 2001).

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 AHP conductance and a decrease in firing rate. (rev. in Powers and Binder 2001). Earlier work indicated that AHP 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 AHP 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 at present no convincing evidence for functionally
important sAHPs in mature motoneurons (Powers and Binder 2001). Further, there is at present 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 (Sawczuk 1993; Musick 1999). 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; Lee and Heckman 1998, 2000; Hsiao et al. 1998; Powers and Binder 2002). 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 Hounsgaard 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 the present study was to consider the potential contribution of the slowly inactivating persistent sodium current (I_{NaP}) to spike-frequency adaptation in rat hypoglossal motoneurons. We used the anticonvulsant phenytoin to reduce the I_{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. Lampl, Schwindt et al. 1998). Phenytoin appeared to be the agent of choice as 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 (rev. in Kuo and Bean 1994). As expected, adding phenytoin (≥ 50 μM) 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 mV to 0 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 et al. 1999; Powers and Binder 2001).

METHODS

Slice preparation

These experiments were carried out with protocol approval from the IACUC Committee at the University of Washington. Rat hypoglossal motoneurons were studied in 250 - 400 μm thick brainstem slices obtained from 12- to 22-day-old Sprague-Dawley rats as described in prior publications from our laboratory (Sawczuk et al. 1995b; Powers and Binder 2003). Following induction of anesthesia with an intramuscular injection of a mixture of ketamine (68 mg kg⁻¹) and xylazine (4 mg kg⁻¹), the animals were decapitated and a section of brainstem was removed and glued to a Plexiglas tray filled with cooled, modified, artificial cerebrospinal fluid (ACSF). A DSK microslicer was
used to cut a series of transverse slices throughout the length of the hypoglossal nucleus. The slices were transferred to a holding chamber and incubated at room temperature (19-21°C) in the modified ACSF for 30 min, followed by 30 min incubation in standard ACSF.

**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 Ca\(^{2+}\), high Mg\(^{2+}\) solution (Low Ca-ACSF (in mM): 132 NaCl, 3 KCl, 1.25 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), 5 MgCl\(_2\), 1 CaCl\(_2\) 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 substituted for NaCl, and concentrations of MgCl\(_2\) and CaCl\(_2\) 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 ASCF was identical to that of the S-ACSF except that 132 mM NaCl was substituted for sucrose. The pH of the ASCF solutions ranged from 7.3 – 7.4 and their measured osmolalities ranged from 310 – 320 mOsm.

To study the entire ensemble of whole-cell currents, we used a patch recording solution containing (mM): 146 KCH\(_3\)SO\(_4\), 5 KCl, 2 MgCl\(_2\), 2 EGTA, 10 MOPS, 2 Na\(_2\)ATP, 0.2 Na\(_3\)GTP. KOH / HCl were added to bring the pH to 7.2. The osmolality of this solution was 310 mOsm. Patch solution aliquots were stored -20°C until time of use.

To minimize the contribution of potassium currents, the pipette solution was composed of (mM): 100 CsCl, 20 TEACl, 5 MgCl\(_2\), 2 BAPTA, 10 HEPES, 5 Na\(_2\)ATP, 0.5 Na\(_3\)GTP, 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-aminopyridine (4-AP), 10 mM tetraethylammonium chloride (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 up to 100 μM.

**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, Inc.).

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 mV 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 perfusion solution (i.e., control, phenytoin or wash), 15-30 minutes were allowed to elapse before obtaining additional recordings.

**Intracellular recordings with sharp electrodes**

Intracellular recordings were made from hypoglossal motoneurons with glass micropipettes filled with 3 M KCl (resistances of 30 to 80 MΩ). We used an Axon Instruments Axoclamp 2B amplifier and digitized the records at 10 kHz using an Instrutech A/D board connected to a Macintosh PowerPC. Motoneuron identity was based on anatomical location and the similarity of its intrinsic properties to our previous samples (Sawczuk et al. 1995b, 1997; Poliakov et al. 1996; Powers and Binder 2002). Following impalement of cells with membrane potentials >60 mV, we measured rheobase by injecting 50 ms current pulses, input resistance by injecting 500 ms pulses, and the relationship between firing frequency and injected current (f-I relation) with a series of 1 s current steps of different magnitude. Spike-frequency adaptation was measured by injecting a series of 30 s constant-current steps into the motoneurons. We generally used several different current levels (up to 12) and completed as many as 50 trials in a single cell, including trials before, during and after phenytoin (50 or 100 μM) was added to the bathing solution. As was the case for the whole-cell protocol described above, following a change in the bathing solution (i.e., control, phenytoin or wash), we waited 15-30 minutes before obtaining additional recordings.
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 \( f_t \) and the average firing rate over the last second of discharge (\( f_f \)). The form of the single exponential fit was:

\[
\text{Firing Rate} = f_f + K_L \ast \exp \left(-\frac{t}{T_L}\right)
\]

where \( K_L \) is the magnitude and \( T_L \) 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 \( f_f \)) 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 (Spielmann et al. 1993; Sawczuk et al. 1995b). 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 first of three points where the rate of change of membrane potential exceeded 10 mV/ms) to the peak amplitude.

Statistical comparisons between two different conditions (i.e., control and phenytoin) were based on unpaired t-tests, using the Bonferroni/Dunn correction for multiple comparisons. Statistical comparisons were also made for a number of measures using an ANOVA with three
treatment groups (control, 50 μM phenytoin and 100 μM phenytoin). For group comparisons with small sample sizes, the non-parametric Wilcoxon Signed Rank Test was used.

RESULTS

Effects of phenytoin of persistent inward currents

We studied the effects of phenytoin on persistent inward currents (PICs) in 20 hypoglossal motoneurons recorded in brainstem slices taken from rats aged 12–22 days. 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 Ca²⁺ 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 mV 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 (-55 to -35 mV) appears to be completely blocked by the phenytoin. We have previously shown that much of this component of the PIC is TTX-sensitive in hypoglossal motoneurons, presumably mediated by persistent Na⁺ channels (Powers and Binder 2003). Washing out the phenytoin (Fig. 1A; blue trace) resulted in a marked recovery of the PIC, particularly the lower-voltage activated component, which could be reversed by re-applying the phenytoin (Fig. 1A; green trace). A phenytoin concentration of 50 μM produced a much smaller and quite variable reduction in the PICs (Fig. 1B)
Panel C of figure 1 shows the leak-subtracted current-voltage plots derived from the responses of another hypoglossal motoneuron to voltage-clamp ramps identical to those used in the experiment illustrated in panel A of the figure. However, in this case, the CaCl₂ component of the perfusate was replaced with MnCl₂. In the absence 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 μM phenytoin to the bathing solution blocked nearly all of the persistent Na⁺ current (red trace). This figure also demonstrates 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.
Fig. 1. Phenytoin reduces the persistent inward currents (PICs) in rat 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, TEA and 4-AP. The 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). B. Same protocol as in A, but with 50 μM phenytoin added to the bath. C. Same protocol as in A, with Mn²⁺ substituted for Ca²⁺ in the bathing solution: adding 100 μM phenytoin (red trace) blocked nearly all of the persistent Na⁺ current (black trace).
Effects of phenytoin of spike-frequency adaptation

We studied the effects of phenytoin (20-100 μM) 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 μM (n=70 trials) and/or 100 μM 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 panel A of the figure, 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, the red trace after 100 μM 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). Further, the entire time courses of the later phases of the spike-frequency adaptation were virtually identical. Although phenytoin increased the rheobase current (0.25 nA +/- .11 S.E.M; 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.
Fig. 2. Effects of phenytoin on spike-frequency adaptation and spike height. A. superimposed firing rate versus time plots for the same cell in response to identical current steps of 1.4 nA: the black trace was obtained under control conditions, the red trace after 100 μM phenytoin was added to the bathing solution. B. plots of spike height versus time during the repetitive firing trials shown in A: black trace - control; red trace - phenytoin. C. From left to right: the first spike from each trial, followed by averages of 10 spikes taken at the end of 1s, 5s, and 30s after current onset during both the control (black) and phenytoin (red) trials shown above.

The graphs in figure 3 displays the relationship between the total amount of spike-frequency adaptation (i.e., $f_i - f_l$) 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 (30s) of repetitive firing. The open circles in the graphs represent the control trials, filled circles represent the trials recorded in 50 μM phenytoin and the asterisks show the data for 100 μM 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.
Fig. 3. Effects of phenytoin on the relationships between firing rate and spike-frequency adaptation during 30-s repetitive firing trials. **A.** Total spike-frequency adaptation (i.e., $f_i - f_f$) versus initial firing rate for seven rat hypoglossal motoneurons. **B.** Initial adaptation versus initial firing rate for same cells. **C.** Later phases of adaptation (i.e., early + late) versus the transition firing rate ($f_t$; firing rate after initial phase of adaptation) for same cells. Open circles represent control trials ($n=115$), filled circles represent trials in 50 µM phenytoin ($n=70$) and asterisks represent trials in 100 µM phenytoin ($n=43$).
Thus, although treatment with 50 or 100 μM phenytoin reduces the persistent sodium current in rat hypoglossal motoneurons (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 (Sawczuk 1993; Musick 1999). 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).

Part B of Fig. 2 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. However, during the late phase of adaptation, there is a greater reduction in spike height in the phenytoin trial. In figure 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 1s, 5s and 30s 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 relationship between spike height and firing rate from the end of the initial adaptation (ft) 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 ± 0.64 mV/imp/s under control conditions (n=115 trials), 1.16 ± 0.46 mV/imp/s after the application of 50 μM phenytoin (n=70 trials) and 1.38 ± 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 $f_t$ and $f_f$ as a function of $f_t$. 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 (open circles: $r=0.58; p<0.001$), for the 50 μM phenytoin trials (filled circles: $r=0.73; p<0.001$) and for the 100 μM phenytoin trials (asterisks: $r=0.63; p<0.001$) when considered separately. However, as suggested by the example presented in Fig. 2B, the change in spike height during the later phases of adaptation was significantly greater in phenytoin than in control (ANOVA, F= 4.34, p < 0.05). The average change in spike height was 16.1 mV under control conditions, 20.6 mV for the 50 μM phenytoin trials and 20.1 mV for the 100 μM phenytoin trials. The
change in height was significantly greater in the 50 μM phenytoin trials than in the control trials (p < 0.01).

**Fig. 4.** Effects of phenytoin on the change in spike height during sustained, repetitive firing. The change in spike height from the end of the initial phase of adaptation (f_t, Transition firing rate) to the end of the 30-s trials is plotted as a function of f_t. There was a strong correlation between the change in spike height during the later phases of adaptation and the transition firing rate for all of the data, but the change in spike height was greater in the presence of phenytoin. Data are derived from the same trials presented in Fig. 3. Open circles represent control trials (n=115), filled circles represent trials in 50 μM phenytoin (n=70) and asterisks represent trials in 100 μM phenytoin (n=43).

**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 had been demonstrated 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). Further, based simply of 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 lower firing rates (Powers et al. 1999; Powers and
But, we were wrong. At doses of \( \geq 50 \, \mu M \), phenytoin does, indeed decrease persistent inward currents in rat hypoglossal motoneurons. Further, phenytoin often enhanced the decline in spike amplitude that occurs during sustained repetitive firing. This finding is consistent with previous work showing that phenytoin acts on \( \text{Na}^+ \) channels in a use-dependent manner: when studied with trains of action potentials, the blockade is more pronounced late in the train (rev. in Kuo and Bean 1994). However, the phenytoin treatment did not appear to affect either the magnitude or the time course of spike-frequency adaptation in these motoneurons.

This is not the first time that the robust phenomenon of spike-frequency adaptation in motoneurons has confounded our expectations. On ‘first principles’, one would predict that decreasing a source of depolarizing current as we have done in the present study with phenytoin should result in 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 net outward current as we did in an earlier study by blocking the \( \text{Na}^+\text{-K}^+ \) pump with oubain should result in an increase in firing rate (i.e., a decrease in the later phases of adaptation; Sawczuk et al. 1997). But, in both studies, the magnitude and time course of spike-frequency adaptation proved to be unassailable.

One can only conclude from these results, as we have posited previously (Powers et al. 1999), that several of the membrane channels involved in generating action potentials can and do contribute to spike-frequency adaptation: Changing the contribution of one set of channels to spike-frequency adaptation is compensated for by an increased role of another set of channels. Thus, the finding that a reduction in persistent sodium current did not alter spike-frequency adaptation does not exclude its contribution to the
phenomenon. One possibility is that the decrease in spike height during the later phases of adaptation leads to a reduction in calcium entry during the spike and decreased activation of the calcium-activated potassium conductance mediating the mAHP. The relative balance of inward and outward currents could thus be unaffected by phenytoin application.

It may only be possible to significantly alter the time course of the later phases of adaptation by applying a specific ‘cocktail’ of channel blockers. The challenge would be to provide a mixture of blockers that can significantly alter spike-frequency adaptation without completely eliminating 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 (rev. 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 (Sawczuk et al. 1995, 1997; Powers et al.
1999) are a specialized feature of hypoglossal motoneurons to accommodate their essential contributions to respiration and feeding.

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