CONTRIBUTION OF OUTWARD CURRENTS TO SPIKE-FREQUENCY ADAPTATION IN HYPOGLOSSAL MOTONEURONS OF THE RAT

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Abstract

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Contribution of outward currents to spike-frequency adaptation in hypoglossal motoneurons of the rat. J. Neurophysiol. 78: 2246–2253, 1997. Spike-frequency adaptation has been attributed to the actions of several different membrane currents. In this study, we assess the contributions of two of these currents: the net outward current generated by the electrogenic Na⁺-K⁺ pump and the outward current that flows through Ca²⁺-activated K⁺ channels. In recordings made from hypoglossal motoneurons in slices of rat brain stem, we found that bath application of a 4–20 μM ouabain solution produced a partial block of Na⁺-K⁺ pump activity as evidenced by a marked reduction in the postdischarge hyperpolarization that follows a period of sustained discharge. However, we observed no significant change in either the initial, early, or late phases of spike-frequency adaptation in the presence of ouabain. Adaptation also has been related to increases in the duration and magnitude of the medium-duration afterhyperpolarization (mAHP) mediated by Ca²⁺-activated K⁺ channels. When we replaced the 2 mM Ca²⁺ in the bathing solution with Mn²⁺, there was a significant decrease in the amplitude of the mAHP after a spike. The decrease in mAHP amplitude resulted in a decrease in the magnitude of the initial phase of spike-frequency adaptation as has been reported previously by others. However, quite unexpectedly we also found that reducing the mAHP resulted in a dramatic increase in the early time course (1–2 s) of adaptation (Lorenzon and Sjoholm 1973). This increase in mAHP amplitude would support the hypothesis that the initial and later (i.e., early and late) phases of spike-frequency adaptation are mediated by different cellular mechanisms.

INTRODUCTION

In response to a constant-current stimulus, motoneurons display a pronounced reduction in firing rate during the first second of discharge followed by a more gradual decline in frequency that continues during tens of seconds or even several minutes (Granit et al. 1963; Kernell 1965a,b; Kernell and Monster 1982; reviewed in Binder et al. 1996). The time course of this spike-frequency adaptation in rat hypoglossal motoneurons generally has three distinct phases: initial, early, and late (Sawczuk et al. 1995). In these cells, initial adaptation is a linear function of time and is complete after several minutes (Granit et al. 1963; Kernell 1965a,b; Kernell and Monster 1982; reviewed in Binder et al. 1996). The time proposed as contributors to the later phases of adaptation in several minutes (Granit et al. 1963; Kernell 1965a,b; Kernell and Monster 1982; reviewed in Binder et al. 1996). The time course of this spike-frequency adaptation in rat hypoglossal motoneurons and several other types of neurons (Table 1). These include slow sodium channel inactivation (Basarsky and French 1991; Fleidervish and Gutnick 1996; Fleidervish et al. 1996; reviewed in Jack et al. 1975), a slowly activated potassium conductance (reviewed in Jack et al. 1975), activ-
TABLE 1. Spike-frequency adaptation in rat hypoglossal motoneurons

<table>
<thead>
<tr>
<th>Phase</th>
<th>Relative Magnitude, %</th>
<th>Time Course, s</th>
<th>Possible Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial adaptation</td>
<td>74 ± 27</td>
<td>duration = 0.038 ± 0.011</td>
<td>mAHP summation, m Na⁺ channel inactivation, inward rectifier (Iₖ) deactivation</td>
</tr>
<tr>
<td>Early adaptation</td>
<td>13 ± 16</td>
<td>τ = 0.24 ± 0.37</td>
<td>Persistent Na⁺ current inactivation, sAHP, m-type K⁺ current, slow Na⁺ channel inactivation, delayed rectifier inactivation, N⁺K⁺ ATPase activity, saturation of Ca²⁺ sequestering systems</td>
</tr>
<tr>
<td>Late adaptation</td>
<td>14 ± 10</td>
<td>τ = 23 ± 19</td>
<td>same as Early Adaptation</td>
</tr>
</tbody>
</table>

Data and nomenclature from Sawczuk et al. (1995). Relative magnitude values are means ± SD. mAHP, medium-duration after hyperpolarization; SAHP, slow AHP. Mechanism references: ¹ Baldissera and Gustaffson 1971, Baldissera et al. 1978, Barrett et al. 1980 (cat spinal motoneurons); Krell and Sjöholm 1973 (cat spinal motoneurons and neuron model); Baldissera and Gustaffson 1974 (neuron model); ² Schwindt and Crill 1982 (cat spinal motoneurons); ³ Schwindt et al. 1988a, España et al. 1987 (cat cortical neurons); ⁴ Nishimura et al. 1989 (guinea pig facial motoneurons); Fleidervish and Gutnick 1996, Fleidervish et al. 1996 (mouse and guinea pig cortex); ⁵ Safronov and Vogel 1996 (neonatal rat spinal motoneurons); ⁶ e.g., Marrion 1993 (cultured frog sympathetic neurons); ⁷ Basarsi and French 1989 (cockroach sensory neuron); Fleidervish and Gutnick 1996, Fleidervish et al. 1996 (mouse and guinea pig cortex); ⁸ e.g., Marom and Levitan 1994 (rat brain K channels expressed in Xenopus oocytes); ⁹ Krell and Monster 1982 (cat spinal motoneurons); French 1989 (cockroach sensory neuron); ¹¹ Sawczuk et al. 1995 (rat hypoglossal motoneurons).

ity of the Na⁺-K⁺ pump (French 1989; Krell and Monster 1982; Sokolove and Cook 1971; reviewed in Jack et al. 1975), continued increases in the amplitude of the mAHP (Hounsgaard et al. 1988), as well as the activation of a sAHP with an even slower time course (cf. Schwindt et al. 1988b). It is likely that more than one of these hypothesized mechanisms contribute to the later phases of adaptation, even within a given cell type. For example, recent evidence suggests that both slow sodium channel inactivation (Fleidervish et al. 1996) and the activation of a slow afterhyperpolarization (Schwindt et al. 1988b) can contribute to the later phases of adaptation in mammalian neocortical neurons.

The goal of this study was to assess the contributions of two outward currents that have been proposed as contributors to spike-frequency adaptation: the net outward current generated by the electrogenic Na⁺-K⁺ pump and the outward current that flows through Ca²⁺-activated K⁺ channels. We found that bath application of ouabain to slices of the rat brain stem produced a partial block of Na⁺-K⁺ pump activity but no significant change in any of the three phases of spike-frequency adaptation. When we blocked Ca²⁺-activated K⁺ channels with Mn²⁺, there was a significant decrease in the amplitude of the mAHP after a spike and a decrease in the magnitude of the initial phase of spike-frequency adaptation. There was also a dramatic increase in the magnitude of both the early and late phases of adaptation. All of these changes could be reversed by restoring the normal Ca²⁺ concentration in the bath.

Preliminary accounts of some of these data have been previously presented (Sawczuk and Binder 1992–1994).

METHODS

Our preparation of rat brain stem slices and techniques for making intracellular recordings from hypoglossal motoneurons were identical to those detailed in two recent papers from this laboratory (Poliakov et al. 1996; Sawczuk et al. 1995). Briefly, the brain stems were removed from young, adult Sprague-Dawley rats (3–8 wk old) after anesthesia by an intramuscular injection of a mixture of ketamine and xylazine (68 and 4 mg/kg, respectively). Slices (400 μm) were cut and maintained in cooled, oxygenated artificial sucrose-cerebral spinal fluid (S-ACSF) composed of (in mM) 220 sucrose, 2.0 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 2.0 MgCl₂, 2.0 CaCl₂, and 10 glucose. Final incubation and electrical recordings were done at room temperature in normal, artificial cerebral spinal fluid (N-ACSF), which differs from S-ACSF by excluding the sucrose and including 126 mM NaCl. The N-ACSF was maintained at pH 7.4 and oxygenated by gentle bubbling with 5% CO₂-95% O₂ (carbogen) throughout the experiment.

Experimental protocols

Intracellular recordings were made from hypoglossal motoneurons with glass micropipettes filled with 3 M KCl (resistances of 30–80 MΩ). Motoneuron identity was based on the similarity of its intrinsic properties to the published results of previous investigators (Haddad et al. 1990; Mosfeldt-Laursen and Rekling 1989; Nunez-Abadez et al. 1993; Poliakov et al. 1996; Sawczuk et al. 1995; Viana et al. 1990, 1993). After 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. We measured the time required for the membrane potential to return to 1/e of its maximum value after the depolarizing pulses to estimate the membrane time constant (τᵣₑ) (cf. Mosfeldt-Laursen and Rekling 1989). Spike-frequency adaptation was measured by injecting a series of 60-s constant-current steps into the motoneurons. We generally used several different current levels and completed as many as 15 trials in a single cell, including trials before, during, and after modifications were made in the bathing solution. After each adaptation trial, rheobase and spike height were remeasured.

Ouabain block of Na⁺-K⁺ ATPase

To assess the possible contribution of Na⁺-K⁺ pump activity to spike-frequency adaptation, N-ACSF containing from 4 to 20 μM ouabain was introduced into the chamber after recording an initial series of adaptation trials at several different current strengths. (Ouabain concentrations >20 μM generally resulted in broad, short spikes and eventual cessation of repetitive discharge. In view of this toxic effect of ouabain, trials only were accepted for analysis if regular discharge was observed throughout the 60-s trials). At least 5 min elapsed before restesting the responses of the cell to another series of 60-s trials. Subsequently, the ouabain solution was washed-out for ≥10 min, and if the cell’s discharge remained robust, another series of adaptation trials was performed. The efficacy of the ouabain in blocking the pump was determined off-line by comparing the postdischarge hyperpolarization (PDH) after 60-s trials before, during, and after the ouabain application. The PDH...
was referred to the cell’s resting membrane potential just before the onset of each period of repetitive firing.

Manganese block of calcium channels

We substituted 2 mM Mn\(^{2+}\) for the 2 mM Ca\(^{2+}\) in the N-ACSF bath solution to block Ca\(^{2+}\)-activated K\(^{+}\) channels. The NaH\(_2\)PO\(_4\) also was eliminated from the bathing solution to prevent chelation of the Mn\(^{2+}\). The efficacy of the Mn\(^{2+}\)-block was assessed by monitoring the reduction in amplitude of the mAHP after action potentials evoked by short current pulses (cf. Fig. 3A). Multiple trials were performed in the presence of Mn\(^{2+}\), both at current levels used in the preceding control trials and whenever possible at current levels that reproduced the initial firing frequencies generated in the control trials. Wash-out of the Mn\(^{2+}\) was monitored by observing the restoration of the mAHP amplitude to its control value. Post-Mn\(^{2+}\) adaptation trials were performed whenever the cell remained viable.

Data analysis

Our procedures for off-line analysis of motoneuron properties, including repetitive firing behavior and spike-frequency adaptation, have been detailed in several recent publications from this laboratory (Poliakov et al. 1996; Powers and Binder 1995; Powers et al. 1993; Sawczuk et al. 1995). Cells were selected for detailed analysis based on the guidelines established by Mosfeldt-Laursen and Rekling (1989): maintained resting membrane potentials \(> 55\) mV; input resistances \(> 5\) MΩ; and time constants \(> 2\) ms.

Analysis of spike-frequency adaptation as defined in Sawczuk et al. (1995) was performed on every long 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. 1). The subsequent exponential decline in firing rate was measured as the difference between \(f_i\) and the average firing rate during the last second of discharge (\(f_{AV}\)). First, a single exponential fit was attempted that had the form

\[
Firing Rate = f_0 + K_0 \cdot \exp(-t/\tau_L)
\]

where \(K_0\) is the magnitude and \(\tau_L\) 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 \(f_1\)) 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. 1995; Spielmann et al. 1993). The inset in Fig. 1 illustrates this method and shows that this firing rate-time plot was best described by a double exponential function. Thus the entire time course of adaptation for the trial in Fig. 1 consisted of three phases: an initial linear decline in rate with a slope of \(\frac{1.3 \times 10^{-4}}{\text{imp/s}}\); an early exponential decline (\(\tau_E = 0.2\) s); and a late, slow exponential decline (\(\tau_L = 15.0\) s).

Paired \(t\)-tests were used to compare the motoneuron responses to identical 60-s current steps before and during the application of ouabain (postouabain trials were generally not available). Since the replacement of Ca\(^{2+}\) with Mn\(^{2+}\) changes the \(f-I\) relation (e.g., Nishimura et al. 1989; Viana et al. 1993) (RESULTS), the responses to a range of current step amplitudes were compared before, during, and after Mn\(^{2+}\) using a one-way analysis of variance (ANOVA). Statistical comparisons between two different conditions (i.e., Pre-Mn\(^{2+}\) vs. Mn\(^{2+}\), Mn\(^{2+}\) vs. Post-Mn\(^{2+}\), etc.) were based on unpaired \(t\)-tests, using the Bonferroni/Dunn correction for multiple comparisons.

**FIG. 1.** Characteristics of the time course of spike-frequency adaptation in rat hypoglossal motoneurons. Gray trace shows the instantaneous firing rate of a cell in response to a 60-s suprathreshold step of injected current. \(f_0\) is the instantaneous frequency calculated from the reciprocal of the 1st interspike interval, \(f_i\) is the frequency attained at the end of the initial (linear) phase of spike-frequency adaptation, and \(f_t\) is the average firing frequency during the last second of discharge. Black line is a double exponential fit to the time course of firing frequency from \(f_0\) to \(f_t\), obtained by “peeling off” the longest exponential fit and fitting a 2nd exponential curve to the remainder (cf. METHODS). Inset: expanded view of the 1st 2 s of discharge illustrates that a single exponential curve does not fit the early time course of firing rate immediately after initial adaptation.

**RESULTS**

Recordings from 15 rat hypoglossal motoneurons were accepted for analysis based on their ability to produce steady, repetitive discharge both before and during alteration of outward currents. All but three of these cells were part of the database presented in Sawczuk et al. (1995). Unless otherwise stated, all trials followed the protocol outlined in METHODS.

**Effects of ouabain on spike-frequency adaptation**

Spike-frequency adaptation was studied before, during, and after bath application of ouabain at concentrations of 4–20 μM. Reduction of the amplitude of the membrane hyperpolarization after 60 s of discharge (PDH) was the criterion used to indicate successful block of pump activity (cf. METHODS). Seven cells were accepted for analysis based on ouabain-induced reduction of the PDH >2 mV. There was a strong correlation between the percentage of PDH reduction (measured at 500 ms after current offset) and the concentration of ouabain (−4.1% reduction per μM ouabain; \(r = 0.81, P < 0.05\)). However, the ouabain never completely eliminated the PDH as previously reported for other types of neurons (Parker et al. 1996; Schwindt et al. 1988b, 1989).

The addition of ouabain to the bathing solution had no significant effect on spike-frequency adaptation during 60 s of repetitive discharge for any of the seven cells. The mean magnitude of initial adaptation (\(f_i - f_o\), cf. Fig. 1) was nearly identical before and during ouabain application (104.9 ± 36.8 imp/s and 104.0 ± 30.2 imp/s, respectively; means ± SD). The later phases of spike-frequency adaptation were similarly unaffected by the ouabain. Figure 2A demonstrates the similarity of the firing rate-time plots for...
a motoneuron in response to the same 60-s current pulse before and during ouabain application. Figure 2B shows the reduction of the PDH in this cell with bath application of ouabain. The 8-mV reduction in the PDH of this cell was ~31% of the total PDH magnitude. The magnitude of spike-frequency adaptation after the initial phase \((f_t - f_i, \text{ cf. Fig. 1})\) was not significantly affected by the application of ouabain \((17.7 \pm 9.6 \text{ imp/s} \text{ before vs. } 17.6 \pm 8.3 \text{ imp/s during ouabain, paired } t = 0.06, P = 0.96)\) nor was the magnitude of the early phase of this adaptation \((6.6 \pm 5.4 \text{ imp/s before ouabain vs. } 5.2 \pm 3.8 \text{ imp/s with ouabain, paired } t = 0.68, P = 0.52)\).

Due to the progressive toxicity of ouabain \(\text{(see METHODS)}\), we could obtain only acceptable postouabain recordings from three of the seven cells studied. In these cells, the characteristics of spike-frequency adaptation after ouabain wash-out were quite similar to those obtained before and during the ouabain treatment. All of these results indicate that membrane hyperpolarization due to activation of the \(\text{Na}^+\text{-K}^+\) pump does not make a significant contribution to spike-frequency adaptation in rat hypoglossal motoneurons.

**Manganese block of calcium channels**

Ten motoneurons were studied before and after the substitution of \(\text{Mn}^{2+}\) for \(\text{Ca}^{2+}\) in the bathing solution. Of these, eight cells were accepted for analysis. The remaining two cells produced acceptable discharge before and after the \(\text{Mn}^{2+}\) substitution but would not discharge for more than a few seconds in the presence of \(\text{Mn}^{2+}\). Changes in the mAHP after single action potentials were monitored during \(\text{Mn}^{2+}\) wash-in and wash-out and between 60-s trials with \(\text{Mn}^{2+}\) \(\text{(cf. METHODS)}\). Analysis of spike-frequency adaptation in \(\text{Mn}^{2+}\) was limited to trials collected after the amplitude of the mAHP had been reduced to <50% of its control value. Figure 3A shows the loss of the mAHP after a single spike during \(\text{Mn}^{2+}\) wash-in and its return during \(\text{Mn}^{2+}\) wash-out. The marked decrease in the mAHP in the presence of \(\text{Mn}^{2+}\) led to a decrease in the initial phase of spike-frequency adaptation and an increase in the later phases of adaptation. This latter effect is illustrated in Figure 3, \(B-D\), which shows the time course of spike-frequency adaptation at three different current levels before \(\text{(Fig. 3B)}\), during \(\text{(Fig. 3C)}\), and after \(\text{(Fig. 3D)}\) perfusion with the \(\text{Mn}^{2+}\) solution. The total amount of adaptation after the initial phase \((f_t - f_i)\) is clearly larger during \(\text{Mn}^{2+}\) perfusion than it was either before or after in the normal ACSF. This effect of \(\text{Mn}^{2+}\) was significant over the entire sample of trials \(\text{(one-way ANOVA, } P < 0.001)\): the mean magnitudes of the later phases of spike-frequency adaptation \((f_t - f_i)\) were \(9.5 \pm 4.6 \text{ imp/s} \text{ (} n = 16\text{)}\) for the pre-\(\text{Mn}^{2+}\) trials, \(30.8 \pm 7.9 \text{ imp/s} \text{ (} n = 13\text{)}\) with \(\text{Mn}^{2+}\), and \(12.3 \pm 3.7 \text{ imp/s} \text{ (} n = 8\text{)}\) after \(\text{Mn}^{2+}\) wash-out. The values obtained during \(\text{Mn}^{2+}\) perfusion were significantly different from those obtained either before or after \(\text{Mn}^{2+}\) perfusion \(\text{(} P < 0.001\text{)}\), whereas those obtained pre- and post-\(\text{Mn}^{2+}\) did not differ significantly from one another \(\text{(} P = 0.28\text{)}\). In a few cases, we also recorded 60-s trials of repetitive discharge as the \(\text{Mn}^{2+}\) solution was being washed in or out, when the mAHP was blocked incompletely. We found that the increase in the later phases of spike-frequency adaptation does not appear until the mAHP is reduced by \(\geq 50\%\).

At each level of injected current, the substitution of \(\text{Mn}^{2+}\) for \(\text{Ca}^{2+}\) led to an increase in firing rate shortly after the onset of the current step, as has been reported previously by others \(\text{e.g., Nishimura et al. 1989; Viana et al. 1993)}\). Thus to study the changes in spike-frequency adaptation produced by the \(\text{Mn}^{2+}\) at the same firing rate, we generally used a lower range of injected current levels during \(\text{Mn}^{2+}\) perfusion than during perfusion in control ACSF. The mean values of the initial firing rates \(\text{i.e., the 1st interspike interval})\) were similar in \(\text{Mn}^{2+}\) and control solutions \(\text{(pre-} \text{Mn}^{2+} : 84.9 \pm 40.4 \text{ imp/s, post-} \text{Mn}^{2+} : 86.7 \pm 29.6 \text{ imp/s)}\), although a wider range of initial firing rates were observed in control ACSF. Figure 4A shows the relation between the initial firing rate \((f_t)\) and the extent of initial adaptation \((f_t - f_i)\) before \((\odot)\), during \((\bigcirc)\), and after \((\Box)\) the substitution of \(\text{Mn}^{2+}\) for \(\text{Ca}^{2+}\). For matched initial firing rates, there was less initial adaptation in the presence of \(\text{Mn}^{2+}\) than in the control solution. In contrast, for matched initial interval firing rates, the total amount of adaptation \((f_t - f_i)\) was similar in \(\text{Mn}^{2+}\) and control conditions \(\text{(Fig. 4B)}\). As a result, the initial phase of adaptation was a smaller percentage of the total amount of adaptation in \(\text{Mn}^{2+}\) than in the control conditions \(\text{(pre-} \text{Mn}^{2+} : 76.2 \pm 23.3\% ; \text{Mn}^{2+} : 57.3 \pm 7.1\%; \text{post-} \text{Mn}^{2+} : 76.8 \pm 16.7\% ; \text{Mn}^{2+} \text{significantly less than pre-} \text{Mn}^{2+} , P < 0.01\text{).}
The decrease in initial adaptation during Mn^{2+} perfusion led to a higher firing rate at the beginning of the later phases of adaptation ($f_0$). The increase in the later phases of adaptation might have been simply a consequence of the increase in $f_0$, because the magnitude of a given phase of adaptation is correlated positively with the firing rate at the beginning of that phase (Sawczuk et al. 1995). However, Fig. 4C illustrates that even for matched values of $f_0$, the subsequent phases of adaptation were increased in the presence of Mn^{2+}.

Despite the dramatic increase in the later phases of adaptation during Mn^{2+} perfusion, the time course of adaptation was similar to that observed in the control ACSF. As described above (see METHODS), the time course of adaptation after the initial phase (i.e., from $f_0$ to $f_1$) generally could be fit by the sum of two exponential functions, which we have taken to represent early and late phases of adaptation (Sawczuk et al. 1995). Perfusion with Mn^{2+} did not significantly affect either the time constants or the relative magnitudes of these two phases. The mean time constant of the early phase of adaptation ($\tau_E$) was $0.28 \pm 0.09$ s ($n = 10$) and that of the late phase ($\tau_L$) was $13.44 \pm 8.00$ s ($n = 11$) during Mn^{2+} perfusion [compared with pre-Mn^{2+}: $\tau_E = 0.27 \pm 0.13$ (n = 12) and $\tau_L = 16.44 \pm 7.83$ s (n = 16), and post-Mn^{2+}: $\tau_E = 0.26 \pm 0.16$ (n = 5) and $\tau_L = 10.96 \pm 5.36$ s (n = 8)]. The early phase of adaptation accounted for $34.0 \pm 19\%$ of the decrease in firing rate from $f_0$ to $f_1$ during Mn^{2+} perfusion [compared with pre-Mn^{2+}: $40.2 \pm 14.0\%$ (n = 16) and post-Mn^{2+}: $21.6 \pm 27.4\%$ (n = 8)].

**Discussion**

The intent of this study was to examine the contribution of two different types of outward currents to the spike-frequency adaptation of rat hypoglossal motoneurons: the net outward current mediated by activity of the Na^{+}–K^{+} pump and the outward currents mediated by Ca^{2+}-activated K^{+} channels. We found that bath application of a 4–20 μM ouabain solution produced a partial block of Na^{+}–K^{+} pump activity as evidenced by a marked reduction in the PDH that follows a period of sustained discharge. However, we observed no significant change in any of the three phases of spike-frequency adaptation in the presence of ouabain.

The Na^{+}–K^{+} pump, which under physiological conditions is thought to be activated primarily by a rise in the internal concentration of Na^{+}, produces a net outward current (cf. Jack et al. 1975). The increase in internal sodium produced by repetitve discharge therefore should lead to activation of the pump, which in turn results in a slow development of a hyperpolarizing current and a decrease in discharge rate. Although there is good evidence that activity of the Na^{+}–K^{+} pump contributes to a slow phase of spike-frequency adaptation in invertebrate mechanoreceptors (French 1989; Sokolove and Cooke 1971), we found that reduction of the pump activity with ouabain did not produce a significant change in any of the three phases of adaptation. We do not believe that this negative result was a consequence of using ineffective concentrations of ouabain, because we often applied concentrations >10 μM which have been reported to inhibit ~70% of the pump ATPase activity in rat brain (Sweedner 1979). In addition, we found a dose-dependent reduction in the amplitude of the PDH; this suggests that ouabain did inhibit pump activity. The eventual toxicity of ouabain (see METHODS) together with the possibility that pump inhibition alters the concentration gradient for K^{+} ions (Schwindt et al. 1988b) makes the effects of ouabain difficult to interpret. Nonetheless, our results certainly do not support a primary contribution of Na^{+}–K^{+} pump activity to spike-frequency adaptation in hypoglossal motoneurons.

Previous studies have indicated that Ca^{2+}-activated K^{+} conductances ($G_{KCa}$) may contribute to spike-frequency ad-
OUTWARD CURRENTS AND SPIKE-FREQUENCY ADAPTATION

The medium-duration hyperpolarization after an action potential (mAHP) is known to increase in magnitude when successive spikes are evoked at intervals shorter than the AHP duration (e.g., Baldissera and Gustaffson 1971; Nishimura et al. 1989). Computer simulations based on motoneuron models indicate that this process of AHP temporal summation appears to contribute to the initial phase of spike-frequency adaptation (e.g., Baldissera and Gustaffson 1974; Kernen and Sjoholm 1973; Powers 1993). Temporal summation of the AHP across successive interspike intervals presumably reflects the fact that the calcium concentration in the vicinity of the $G_{KCa}$ channels does not return to its resting level by the end of the interspike interval. Slow increases in the calcium concentration near the $G_{KCa}$ channels would prolong the increase in $G_{KCa}$ beyond the period of initial adaptation. Such increases in calcium concentration might reflect the saturation of intracellular calcium sequestering systems (Barrett et al. 1980) or release of calcium from intracellular stores triggered either by second messengers (Zhang 1989) or by calcium itself (Sah and McLachlan 1991). Finally, another $G_{KCa}$ conductance that is pharmacologically distinct from that underlying the mAHP recently has been described in mammalian neocortical and hippocampal neurons (Madison and Nicoll 1984; Schwindt et al. 1988b; Storm 1990). This $G_{KCa}$ conductance contributes both to a long-lasting AHP (sAHP) after repetitive discharge and to slow spike-frequency adaptation in these cells.

We found that replacement of Ca$^{2+}$ with Mn$^{2+}$ eliminated the mAHP after single spikes and also reduced the magnitude of the initial phase of spike-frequency adaptation. These results support the hypothesis that initial adaptation is, in part, controlled by conductances underlying the mAHP. However, our failure to eliminate completely initial adaptation by blocking the mAHP suggests that other membrane mechanisms also contribute. One possibility is that the depolarization associated with the onset of repetitive discharge leads to the deactivation of an inward rectifier current ($I_h$), that contributes to the early decline in firing rate (Schwindt et al. 1988a; Spain et al. 1987). Although such currents have been shown to exist in rat hypoglossal motoneurons (Bayliss et al. 1994), there has been no direct demonstration of their contribution to initial adaptation. Rapid changes in the inactivation of the voltage-gated sodium channels responsible for spike initiation also might contribute to initial adaptation. The onset of repetitive discharge in cat spinal motoneurons is associated with an increase in the voltage threshold for spike initiation, presumably reflecting partial inactivation of initial segment sodium channels (Schwindt and Crill 1982).

Our results also indicate that outward currents carried by $G_{KCa}$ channels are not essential for the later phases of spike-frequency adaptation, lending further support to the hypothesis that the initial and later phases of spike-frequency adaptation are mediated by different cellular mechanisms (Sawczuk et al. 1995). The continued presence of the later phases of adaptation after Ca$^{2+}$ replacement with Mn$^{2+}$ could be the result of an incomplete Ca$^{2+}$ block that allowed some residual tissue-bound Ca$^{2+}$ to enter during prolonged repetitive discharge. However, the persistently reduced mAHP after single directly evoked spikes suggests that Ca$^{2+}$ channels are blocked adequately. The relationship between the reduction of the mAHP and the dramatic increase in the later phases of adaptation also suggests that our results are not a consequence of tissue-bound Ca$^{2+}$.

**Alternative mechanisms for the later phases of adaptation**

The later phases of adaptation might be produced by a progressive increase in outward currents, a progressive decrease in inward currents, or some combination of these two mechanisms (Table 1). Possible outward currents include a M current (Adams et al. 1986; Marrion 1993; Storm 1990) and a Na$^+$-activated potassium current (Schwindt et al. 1989). Although it is not yet known if significant M currents are present in rat hypoglossal motoneurons, Na$^+$-activated...
potassium channels have been discovered recently in neonatal rat spinal motoneurons, and their activation during repetitive discharge produces a sAHP in these cells (Safronov and Vogel 1996). However, it is not clear that an sAHP is a prominent feature of mature motoneurons (Nishimura et al. 1989; Vianna et al. 1993; J. R. Musick, R. K. Powers, and M. D. Binder, unpublished).

The late phases of spike-frequency adaptation also might be mediated by a slow inactivation of inward currents (Fleidervish and Gutnick 1996; Fleidervish et al. 1996). Slow inactivation processes in initial segment and somatic sodium channels would be expected to lead to a progressive increase in firing threshold along with changes in spike shape (cf. Schwinding and Crill 1982). Although the firing threshold cannot be accurately measured in current-clamp recordings, late adaptation is associated with profound changes in spike shape (Lindsay et al. 1986; Sawczuk 1993; Sawczuk and Binder 1993). In addition, a persistent sodium current has been described in a number of different types of neurons (e.g., Stafstrom et al. 1985), including rodent motoneurons (Mosfeldt-Laursen and Rekling 1989; Nishimura et al. 1989), and it undergoes slow inactivation (Fleidervish and Gutnick 1996; Fleidervish et al. 1996). A slow decrease in the magnitude of this persistent inward current also would contribute to spike-frequency adaptation.

The process of slow inactivation is a common feature of sodium channels in a variety of excitable tissues (e.g., Brismar 1977; Fox 1976; Featherstone et al. 1996; Howe and Ritchie 1992; Simoncini and Stuhmer 1987), and the increase in early and late adaptation that we observed after the replacement of Ca\(^{2+}\) with Mn\(^{2+}\) are certainly consistent with a contribution of slow sodium inactivation. The reduction of the mAHP should lead to an increase in the mean level of membrane depolarization during the interspike interval, which in turn would be expected to accelerate the development of sodium inactivation. Between-cell variations in the kinetics and steady state voltage dependence of slow inactivation may explain why some rat hypoglossal motoneurons were incapable of prolonged discharge after Ca\(^{2+}\) replacement, as also has been reported for turtle spinal motoneurons (Hounsgaard et al. 1988).

There may be additional mechanisms contributing to the early and late phases of adaptation in the presence of normal levels of extracellular Ca\(^{2+}\). Spike-frequency adaptation in rat hypoglossal motoneurons is associated with an increase in spike duration as well as a decrease in the maximum rate of spike repolarization (Sawczuk 1993; unpublished results), that might reflect slow inactivation of the potassium channels contributing to spike repolarization (e.g., Marom and Levitan 1994). The increase in spike duration could lead to an increase in Ca\(^{2+}\) influx and an associated increase in the mAHP.

It is clear that there are a number of potential mechanisms that might contribute to each of the different temporal phases of spike-frequency adaptation (Table 1). Further, it is likely that different mechanisms may be operating in different types of neurons. Unravelling the relative contributions of these processes to adaptation will probably require a combination of voltage-clamp and computer simulation approaches (e.g., Fleidervish et al. 1996).

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