Ionic Current Model of a Hypoglossal Motoneuron

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Purvis, Liston K. and Robert J. Butera. Ionic current model of a hypoglossal motoneuron. J Neurophysiol 93: 723–733, 2005 doi: 10.1152/jn.00703.2004. We have developed a single-compartment, electrophysiological, hypoglossal motoneuron (HM) model based primarily on experimental data from neonatal rat HMs. The model is able to reproduce the fine features of the HM action potential: the fast afterhyperpolarization, the afterdepolarization, and the medium-duration afterhyperpolarization (mAHP). The model also reproduces the repetitive firing properties seen in neonatal HMs and replicates the neuron’s response to pharmacological experiments. The model was used to study the role of specific ionic currents in HM firing and how variations in the densities of these currents may account for age-dependent changes in excitability seen in HMs. By varying the density of a fast inactivating calcium current, the model alternates between accelerating and adapting firing patterns. Modeling the age-dependent increase in H current density accounts for the decrease in mAHP duration observed experimentally, but does not fully account for the decrease in input resistance. An increase in the density of the voltage-dependent potassium currents and the H current is required to account for the decrease in input resistance. These changes also account for the age-dependent decrease in action potential duration.

INTRODUCTION

The hypoglossal motoneuron (HM) innervates the tongue and along with its roles in mastication and swallowing, the tongue plays an important role in breathing (Bartlett et al. 1990; Lowe 1980; reviewed in Sawczuk and Mosier 2001). The muscle of the tongue contributes to effective breathing by the role of ionic currents in these neurons.

The HM is well suited for modeling because the ionic currents found in rat HMs have been thoroughly studied for over a decade (reviewed in Berger 2000). Numerous ionic currents have been identified in neonatal rat HMs and characterized by voltage-clamp experiments. Besides the sodium and potassium channels that generate the action potential (Haddad et al. 1990; Lape and Nistri 1999, 2001; Powers and Binder 2003; Viana et al. 1993a,b), HMs have several different types of calcium channels (Powers and Binder 2003; Umemiya and Berger 1994, 1995; Viana et al. 1993a) as well as a calcium-dependent potassium current (Lape and Nistri 2000; Sawczuk et al. 1997; Viana et al. 1993b). HMs also include a hyperpolarization-activated cation channel whose density changes with age (Bayliss et al. 1994). Other age-dependent changes that have been found are input resistance (R_N), action potential duration, and firing behavior (Viana et al. 1994, 1995). The firing behavior of neonatal HMs changes from a decrementing firing pattern during the 1st wk postnatal to an incrementing firing pattern during the 2nd wk postnatal (Viana et al. 1995). These changes are investigated in the model by varying the densities of the ionic currents.

Previous modeling work from our laboratory has focused on models of rhythmic activity in a transverse brain slice preparation containing the pre-Bötzinger complex (pBC) (Butera et al. 1999a,b; Del Negro et al. 2001). The pBC is a subregion of the ventrolateral medulla and contains a population of cells that is a critical component of the respiratory rhythm generating network in mammals (Gray et al. 2001; Smith et al. 1991). The hypoglossal nucleus is also included in this transverse slice preparation (Bou-Flores and Berger 2001; Smith et al. 1991), and receives excitatory synaptic input from the pBC (Funk et al. 1993). Recordings from the rootlets of this nucleus are commonly used experimentally as a measure of the output of the network (Del Negro et al. 2001; Smith et al. 1991). A model of the HM is required to model the transformation of the pattern generating rhythm in the pBC to effector motoneurons.

The model is a Hodgkin–Huxley (Hodgkin and Huxley 1952) style, single-compartment, electrophysiological model of a HM from a neonatal rat containing a repertoire of ionic currents. The currents found in our HM model include the fast sodium and delayed-rectifier potassium currents, high- and low-voltage-activated calcium currents, voltage- and calcium-dependent potassium currents, a hyperpolarization-activated cationic current, and a persistent sodium current. The model is composed of these currents with parameters determined from experimental data where possible, along with a simplified model of the dynamics of internal calcium concentration used by other investigators (Bertram 1993; Booth et al. 1997). The model reproduces several characteristics of HMs and replicates the neuron’s response to pharmacological experiments. The model is used to explore the roles of each current in shaping action potential dynamics, and to explore the age-dependent changes seen in HMs.

METHODS

Simulations

Simulations were performed using the interactive differential equation simulation package XPP (Ermentrout 2002). The integration was done using the Dormand–Prince integrator built into XPP. MATLAB (The MathWorks, Natick, MA) was also used for model formulation and data analysis.

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Formulation of model

The model is based on a single-compartment Hodgkin–Huxley formalism. The membrane potential is found using the differential equation

$$\frac{dV}{dt} = \frac{1}{C_m} \left( -\sum I_{\text{ion}} + I_{\text{stim}} \right)$$

where $V$ is the membrane potential (mV), $C_m$ is the whole cell capacitance (nF), $t$ is time (ms), $I_{\text{stim}}$ is the applied stimulus current (nA), and $I_{\text{ion}}$ are the ionic currents listed in the Appendix and have the form

$$I_{\text{ion}} = g(V - E_{\text{ionic}})$$

where $E_{\text{ionic}}$ is the equilibrium reversal potential for the ionic species carried by the current and

$$g = \tilde{g} \, y$$

where $\tilde{g}$ is the maximum conductance of each current and $y$ is the product of one or more gating variables raised to integer powers, as described below.

The dynamics of the conductances of the ionic currents regulated by voltage-dependent activation or inactivation variables are described according to

$$\frac{dx}{dt} = \frac{x_a(V) - x}{\tau_a(V)}$$

$$x_a(V) = \frac{1}{1 + e^{V - \theta_a(V)/\sigma_a}}$$

$$\tau_a(V) = \frac{A}{1 + e^{V - \theta_a(V)/\sigma_a} + B}$$

where $x_a(V)$ is the steady-state voltage-dependent (in)activation function of $x$ and $\tau_a(V)$ is the voltage-dependent time constant. $x_a(V)$ is a sigmoid with a half-(in)activation at $V = \theta_a$ and a slope factor $\sigma_a$. $\tau_a(V)$ is a bell-shaped curve. If no experimental data exist for time constant measurements, or no significant change in the model results from including a scaling factor, and $K_2$ is a decay rate constant that represents calcium uptake and diffusion. A similar model has been used by other investigators in the past (Bertram 1993; Booth et al. 1997). In the model, the calcium-activated potassium (SK) current is the only current whose conductance depends on the internal calcium concentration.

All state variables and initial conditions are listed in Table 1. The maximum conductance and reversal potential for each current, along with various other parameters, are listed in Table 2.

**Model Development**

**Sodium currents**

HMs have a fast sodium current ($I_{NaP}$) that when blocked by tetrodotoxin (TTX) removes the ability of the cell to generate an action potential (Haddad et al. 1990; Lape and Nistri 2001; Viana et al. 1993a). In addition, the voltage-ramp data of Powers and Binder (2003) suggest the existence of a persistent sodium current ($I_{NaP}$) in HMs. Those data show an increase in sodium current with increased ramp speed, and no decrease in sodium current at voltages above −30 mV after application of TTX, which indicates a slowly inactivating sodium current (herein, persistent will mean slowly inactivating). Lape and Nistri (2001) present voltage-clamp data for the sodium current, but do not distinguish between the fast sodium and persistent sodium currents as was done by Rybak et al. (2003). Therefore parameters for the fast sodium current in the model are more depolarized than the data of Lape and Nistri (2001). Because the persistent sodium current has not yet been fully described in HMs, the parameters for this current are based on data from other neurons (Kononenko et al. 2004; Rybak et al. 2003). Kononenko et al. (2004) report a time constant of inactivation of 50–250 ms at the peak of activation. The value for the time constant of inactivation of $I_{NaP}$ chosen for the model is in this range [$\tau_{NaP}(V) = 150$ ms].

**Voltage-dependent potassium currents**

Another current found in rat HMs is a tetraethylammonium (TEA)-sensitive potassium current ($I_K$), the delayed rectifier (Haddad et al. 1990; Lape and Nistri 1999; Viana et al. 1993b). HMs also possess a potassium current that is sensitive to 4-aminoypyridine (4-AP). This current is thought to be the fast transient A-type current (Lape and Nistri 1999; Viana et al. 1993b). Viana et al. (1993b) show a dose-dependent increase in action potential duration with application of TEA and 4-AP in neonatal rat HMs, indicating the importance of both of these currents in shaping the action potential. Parameters for $I_K$ and $I_A$ in the model are based on the voltage-clamp data of Lape and Nistri (1999, 2000), although the time constants used are faster than the reported values to produce an appropriate action potential duration (<2 ms; Viana et al. 1994). The model does not distinguish between (in)activation and de(in)activation as reported for the time constant data of $I_A$ (Lape and Nistri 1999).

**Table 1. Initial conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>$V(0)$</td>
<td>−71.847 mV</td>
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<tr>
<td>$m(0)$</td>
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<tr>
<td>$h(0)$</td>
<td>0.981</td>
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<tr>
<td>$m_{NaP}(0)$</td>
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<tr>
<td>$n(0)$</td>
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<tr>
<td>$m_{AP}(0)$</td>
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<tr>
<td>$h_{AP}(0)$</td>
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<tr>
<td>$m_{H}(0)$</td>
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<tr>
<td>$h_{H}(0)$</td>
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<td>$zSK(0)$</td>
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<tr>
<td>$h_{SR}(0)$</td>
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</tr>
<tr>
<td>$m_{SR}(0)$</td>
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</tr>
<tr>
<td>$<a href="0">Ca^{2+}</a>$</td>
<td>0.0604 μM</td>
</tr>
</tbody>
</table>
Calcium currents

It has been shown that rat HMs have both low-voltage-activated (LVA) and high-voltage-activated (HVA) calcium currents (Powers and Binder 2003; Umemiya and Berger 1994, 1995; Viana et al. 1993a). The LVA calcium current is a T-type current (Umemiya and Berger 1994). The properties of the T-type current were found by characterizing the residual current after application of antagonists to the 3 HVA currents. The 3 HVA currents found in HMs are P-type, N-type, and L-type (Powers and Binder 2003; Umemiya and Berger 1995). Umemiya and Berger (1995) report half-(in)activation and time constant values for these currents found by single-channel recordings. The time constants used in the model are based on these recordings; however, the half-(in)activation values are shifted to a more depolarized value to provide a better fit to whole cell data (Umemiya and Berger 1994; Viana et al. 1993a). The half-activation value for $I_{\text{Na}}$ required a considerable hyperpolarizing shift (55 mV) from the reported value of 25 mV to provide a significant contribution to the total calcium current that is consistent with the whole cell data. The L-type HVA current in HMs has dynamics similar to that of the P-type channel (i.e., noninactivating, high-voltage-activated; Umemiya and Berger 1995). However, the L-type current makes up <10% of the total calcium current (Powers and Binder 2003; Umemiya and Berger 1994). For these reasons, the L-type current is not included in this model.

Calcium-activated potassium current

One important feature of rat HMs is the medium-duration afterhyperpolarization (mAHP) after the action potential (Fig. 1). Previous experimental work has shown that the mAHP in rat HMs is calcium dependent, has a reversal potential near that of potassium, and is blocked by apamin (Lape and Nistri 1999; Sawczuk et al. 1997; Viana et al. 1993b). This indicated the existence of the calcium-activated potassium current, or SK current ($I_{\text{SK}}$). Lape and Nistri (2000) made several important observations by subtracting outward currents measured from voltage-clamp data before and after application of apamin: 1) membrane depolarizations as short as 1 ms produce enough calcium entry to evoke measurable SK currents; 2) the membrane conductance underlying $I_{\text{SK}}$ is voltage independent; 3) apamin does not affect resting membrane potential, suggesting $I_{\text{SK}}$ is not activated at resting calcium concentrations; and 4) the $I-V$ relation indicated an activation region between $-40$ and $-10$ mV. The model uses a noninactivating and voltage-independent conductance that activates with an increase in internal calcium concentration, similar to that used by other researchers (Engel et al. 1999). This current, along with the model of internal calcium, satisfies the observations listed above through:

1) fast activation, 2) no voltage-dependent gating variables, 3) an appropriate half-activation calcium level, and 4) dependency on activation of calcium currents in the $-40$ to $-10$ mV range.

Hyperpolarization-activated cationic current

HM also have a hyperpolarization-activated cationic current ($I_{\text{N}}$) that shows a large increase during postnatal development (Bayliss et al. 1994; Haddad et al. 1990). In neonatal rat HMs, the $H$ current is only about one-tenth the density of that in adult rat HMs (Bayliss et al. 1994). The $H$ current activates much more slowly than the other currents in HMs, with a peak activation time constant $>200$ ms (compared with about 10 ms for the calcium currents).

Passive properties

The membrane capacitance of the model is set to match experimental data (Lape and Nistri 1999; Umemiya and Berger 1994). Resting potential and $R_N$ were set to match experimentally determined values for neonatal rat HMs (Viana et al. 1994) by adjusting the maximum conductances of the currents active at rest. The model’s resting membrane potential is $-72$ mV. This value is within the range measured by many different observers for neonatal HMs (Haddad et al. 1990; Lape and Nistri 2000; Viana et al. 1994). The $R_N$ of neonatal rat HMs is reported to be about 35 $\text{M}\Omega$ by Viana et al. (1994), about 120 $\text{M}\Omega$ by Robinson and Cameron (2000), and about 400 $\text{M}\Omega$ by Lape and Nistri (1999). The large difference in observed $R_N$ is probably attributable in part to the different recording techniques and the use of sharp electrodes (Viana et al. 1994) versus whole cell patch-clamp (Lape and Nistri 1999; Robinson and Cameron 2000). The difference between the 2 values found using whole cell patch-clamp techniques could be explained by the time elapsed before recording $R_N$, as suggested by Robinson and Cameron (2000). The $R_N$ of the model is around 40 $\text{M}\Omega$, closer to that reported by Viana et al. (1994).

Maximum conductances

As stated above, the maximum conductances of the currents active at rest were set to match experimental data for $R_N$ and resting potential. Also, the maximum conductance of $I_{\text{Na}}$, $I_{\text{NaP}}$, $I_{\text{K}}$, $I_{\text{SK}}$, $I_{\text{H}}$, $I_{\text{C}}$, and $I_{\text{ENa}}$ are constant values for these currents found by single-channel techniques and the use of sharp electrodes (Viana et al. 1994) versus whole cell patch-clamp (Lape and Nistri 1999; Robinson and Cameron 2000). The difference between the 2 values found using whole cell patch-clamp techniques could be explained by the time elapsed before recording $R_N$, as suggested by Robinson and Cameron (2000). The $R_N$ of the model is around 40 $\text{M}\Omega$, closer to that reported by Viana et al. (1994).

<table>
<thead>
<tr>
<th>Parameter values</th>
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<tbody>
<tr>
<td>$g_{\text{Na}}$ = 0.7 $\mu$S</td>
</tr>
<tr>
<td>$g_{\text{NaK}}$ = 0.05 $\mu$S</td>
</tr>
<tr>
<td>$g_{\text{K}}$ = 1.3 $\mu$S</td>
</tr>
<tr>
<td>$g_{\text{SK}}$ = 0.005 $\mu$S</td>
</tr>
<tr>
<td>$g_{\text{Na}}$ = 0.1 $\mu$S</td>
</tr>
<tr>
<td>$g_{\text{K}}$ = 0.05 $\mu$S</td>
</tr>
<tr>
<td>$g_{\text{SK}}$ = 0.3 $\mu$S</td>
</tr>
<tr>
<td>$g_{\text{A}}$ = 1.0 $\mu$S</td>
</tr>
<tr>
<td>$g_{\text{Na}}$ = 0.005 $\mu$S</td>
</tr>
<tr>
<td>$E_{\text{Na}}$ = 60 mV</td>
</tr>
<tr>
<td>$E_{\text{K}}$ = $-80$ mV</td>
</tr>
<tr>
<td>$E_{\text{SK}}$ = $-50$ mV</td>
</tr>
<tr>
<td>$E_{\text{ENa}}$ = 40 mV</td>
</tr>
<tr>
<td>$E_{\text{SK}}$ = $-80$ mV</td>
</tr>
<tr>
<td>$E_{\text{ENa}}$ = $-38.8$ mV</td>
</tr>
<tr>
<td>$E_{\text{ENa}}$ = $-0.0005$ $\text{mC}$</td>
</tr>
<tr>
<td>$K_1$ = $0.04$ ms$^{-1}$</td>
</tr>
<tr>
<td>$C_{\text{m}}$ = 0.040 nF</td>
</tr>
</tbody>
</table>

FIG. 1. Action potential. Simulation results of the membrane potential during an action potential showing the afterpotentials seen in a neonatal rat hypoglossal motoneuron (HM). Action potential is evoked by a brief (1-ms) current pulse of 1 nA. Action potential is truncated to emphasize the fast afterhyperpolarization (fAHP), afterdepolarization (ADP), and medium-duration afterhyperpolarization (mAHP) after the spike.
RESULTS

Action potential

A typical action potential produced by the model is shown in Fig. 1. The action potential is elicited by a 1-ms depolarizing current pulse of 1 nA. Following the standard fast depolarization and repolarization, HM action potentials display 3 additional features: a fast afterhyperpolarization (fAHP), an afterdepolarization (ADP), and a medium-duration afterhyperpolarization (mAHP). HM do not fire spontaneously and thus require a stimulus current to elicit an action potential. The stimulus current causes a sudden activation of the sodium currents, which leads to the rapid depolarization of the action potential. The repolarization is caused by a combination of sodium current inactivation, activation of the delayed rectifier \( I_K \), and activation of the fast transient potassium current \( I_A \). If the sodium currents are removed from the model, no action potential is generated. Removing \( I_K \) from the model extends the duration of the action potential from 1.4 to about 4 ms. If \( I_A \) is removed, the action potential duration is increased by about 0.5 ms and the resting potential is increased. Removing \( I_A \) also allows burst firing in the model, like that seen in some very young HMs (Viana et al. 1993a). These 4 currents, in combination with the slower activation of the calcium currents, cause the fAHP. A plot of the behavior of \( I_{Na}, I_{NaP}, I_K, I_A \), and \( I_{leak} \) during an action potential is illustrated in Fig. 2, A and C. Because the conductance of \( I_H \) does not vary significantly over a single action potential, the voltage trace of \( I_H \) is similar in shape to that of \( I_{leak} \) during a single action potential and is not included in this figure. During repetitive firing, however, the magnitude of \( I_H \) does vary from spike to spike.

During the action potential, calcium currents become activated by the large depolarization produced by the sodium current (Fig. 2B). These inward currents cause a large influx of calcium and depolarize the membrane after the cell has repolarized, which leads to the ADP. This ADP is both voltage and calcium dependent. Experiments have shown that the amplitude of the ADP increases by elevating external calcium, after barium application, and by stepping from potentials hyperpolarized to the resting potential (Viana et al. 1993a). The model shows an increase in ADP amplitude by increasing the density of calcium channels. For example, raising \( g_Ca \) by 50% increases the ADP amplitude by 1.8 mV (Fig. 3A). The model also exhibits an increase in ADP amplitude when stepping from hyperpolarized potentials. In Fig. 3B, prepulse currents of 0, −0.1, and −0.2 nA were applied before eliciting an action potential. The increase in ADP height is caused by removal of inactivation of \( I_T \) and \( I_K \).

The mAHP is attributed to the calcium-dependent potassium current \( I_{SK} \). The buildup of internal calcium caused by the inward calcium currents activated during the action potential activates the outward SK current (Fig. 2D). The SK current in the model does not show inactivation, so it remains activated until the internal calcium concentration is decreased. Therefore the time course of decay for \( I_{SK} \) is determined by the depletion rate of calcium from the cell. The depletion rate is modeled in the calcium equation with the decay rate set to match the experimentally measured time course of the mAHP.

Apamin simulation

The importance of the SK current in producing the mAHP is clearly identified by apamin, which selectively blocks SK channels (Blatz and Magleby 1986). Viana et al. (1993b) show that apamin reduces the peak amplitude of the mAHP by 90% in HMs, while having no effect on HM passive properties, the depolarizing phase of the action potential, or the time course of repolarization. Lape and Nistri (2000) also report a complete block of the mAHP in HMs on application of apamin. The model’s response to apamin is simulated by setting the maximum conductance of \( I_{SK} \) to zero (\( g_{SK} = 0 \)), effectively removing the current from the model. Figure 4A illustrates the result of this simulated experiment where a single action potential is elicited by a brief pulse of current. With \( g_{SK} = 0 \), the mAHP’s dependency on the SK current in the model is clearly seen. As reported (Viana et al. 1993b), the mAHP is blocked without affecting the passive properties or the depolarizing/repolarizing phase of the action potential. There is also an increase in ADP height, consistent with experimental findings (Lape and Nistri 2000; Viana et al. 1993b).

Experiments have shown that the HM’s response to a step of current after application of apamin is marked by spike frequency adaptation and a large increase in firing frequency (Lape and Nistri 2000; Viana et al. 1993b). These features are reproduced by the model. Figure 4B is the model’s response to
a step of current with $g_{SK} = 0.03 \mu S$. Using a reduced stimulus amplitude of 0.33 nA, the firing frequency decreases from an initial rate of 48 to 22 Hz after 1 s, and eventually stops firing altogether. The decrease in firing frequency seen here is caused by the slow inactivation of $I_{NaP}$. Without $I_{NaP}$, the firing frequency shows an initial increase (attributed to $I_A$) and quickly reaches a steady state (not shown). Also, if $g_{SK}$ is removed completely (i.e., $g_{SK} = 0$), the model does not show the initial spike frequency adaptation (see DISCUSSION).

Firing properties

NEAR THRESHOLD. When the stimulus is near the threshold for firing, some HMs display 2 features that can be reproduced by the model (Fig. 5). The first feature is spike frequency adaptation leading to a cessation of firing after several spikes (Fig. 5A). The number of spikes before cessation is determined by the stimulus amplitude. A slightly smaller (or larger) stimulus amplitude will cause fewer (or more) spikes before ceasing. The persistent sodium current’s contribution to spike frequency adaptation and AP generation can be seen here. It is the decrease in $I_{NaP}$ amplitude resulting from slow inactivation that slows down the firing rate and leads to cessation of firing. If $I_{NaP}$ is removed from the model, there is no stimulus amplitude that will show adaptation or lead to a cessation of firing once firing has been initiated. The second feature reproduced by the model is delayed excitation (DE), which is a common phenomenon caused by $I_A$ where the onset of the first spike is delayed as a result of the large initial potassium current (Fig. 5B). A small amount of DE can be seen in HM recordings (Lape and Nistri 2000; Viana et al. 1993b).

ADAPTATION. When using a stimulus amplitude above threshold, the repetitive firing behavior of neonatal HMs undergoes significant changes during postnatal development. Neonatal HMs show both spike frequency adaptation and acceleration: 79% of HMs at ages P0–P3 show adaptation and 79% of HMs at ages P8–P15 show acceleration (Viana et al. 1995). For adult HMs, the pattern reverts back to adaptation with 79% of HMs again showing adaptation (Viana et al. 1995). The model is capable of generating spike trains that show both adaptation and acceleration, depending on conductance parameters. Figure 6 illustrates the model’s response to a 200-ms step of

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**FIG. 3. ADP.** A: dependency of the ADP on the low-voltage-activated (LVA) calcium current, $I_T$. A 50% increase in $g_T$ results in a 1.8-mV increase in the height of the ADP. B: voltage dependency of the ADP. Holding potentials of $-72$, $-75$, and $-78$ mV produced by hyperpolarizing prepulses of $0$, $-0.1$, and $-0.2$ nA, respectively. Voltage traces are superimposed and truncated to show the increase in ADP amplitude from baseline membrane potential.

**FIG. 4. Apamin simulation.** Effects of apamin are simulated by reducing the maximum conductance of the SK current. $A$: response of the model to a 1-ms current pulse stimulus, $g_{SK} = 0 \mu S$. ADP is enhanced and the mAHP is removed. $B$: response of the model to an extended current pulse with a reduced amplitude ($I_{stim} = 0.33$ nA), $g_{SK} = 0.03 \mu S$. Arrow indicates stimulus onset.

**FIG. 5. Near threshold firing.** Response of the model to a stimulus amplitude near the threshold for firing action potentials, $I_{stim} = 0.22$ nA. $A$: model shows spike frequency adaptation followed by a cessation of firing. $B$, inset: * in $A$ showing delayed excitation (DE) of the first spike. Arrow indicates stimulus onset.

current. Figure 6A is a typical train of model generated action potentials showing spike frequency adaptation. A small amount of adaptation occurs during the first few spikes, and then a steady-state firing frequency is obtained. Figure 6B is a plot of frequency versus time for 3 different input currents. As the amplitude of the stimulus current is increased, the amount of adaptation substantially increases, whereas the duration of the adaptation (number of spikes occurring before reaching steady state) remains short. This response is similar to that seen in Viana et al. (1995) for the majority of P0–P3 HMs. In HMs that show adaptation, the AHP after the first spike is more depolarized than succeeding AHPs (Viana et al. 1995). The model reproduces this behavior. The initial adaptation is caused by the summation of the SK current, similar to that modeled by Powers et al. (1999). Experimental results show that the initial adaptation is, at least in part, controlled by the SK current (Sawczuk et al. 1997).

ACCELERATION. By changing $g_{SK}$ or $g_N$ (see following text), the model’s response to a step of current is transformed from adaptation to acceleration. Figure 7A is a typical train of model generated action potentials showing spike frequency acceleration. As with the case of adaptation, acceleration occurs during the first few spikes. Figure 7B is a plot of frequency versus time, and shows that the amount of acceleration increases with an increase in stimulus current amplitude. This simulation is similar to the behavior seen in Viana et al. (1995) for the majority of P8–P15 HMs. In contrast to the positive AHP summation seen in adaptation, accelerating HMs show a negative AHP summation (Viana et al. 1995). In the model, the first AHP is more hyperpolarized than succeeding AHPs. The cause of the initial acceleration is addressed in the DISCUSSION.

Age-dependent changes

ADAPTATION VERSUS ACCELERATION. To identify a possible mechanism that explains the variation in firing characteristics during postnatal development, we considered 2 relevant experimental results. First, the calcium-dependent potassium current, $I_{SK}$, plays an important role in the frequency control of HMs (Lape and Nistri 2000; Sawczuk et al. 1997; Viana et al. 1993b). Second, experiments have shown that the contribution of the $T$ current to the total calcium current changes during neonatal development (Umemiya and Berger 1994). Because $I_T$ affects $I_{SK}$ through the buildup of internal calcium concentrations, and $I_{SK}$ affects firing frequency, we hypothesized that the change in density of $I_T$ plays a role in the age-dependent firing properties of HMs. To test this hypothesis, we varied the density of $T$ channels in the model by varying $g_T$ over a 10-fold range and measured changes of the first 2 interspike intervals (ISIs). The ISIs are measured from the peak of one spike to the peak of the following spike. Because adaptation (or acceleration) occurs during the first 3 spikes, only the first and second ISIs are considered. Figure 8A illustrates the effect of varying $g_T$ on the first 2 ISIs. At low values of $g_T$, the first ISI is smaller than the second ISI (i.e., the frequency decreases with time, or adaptation). An increase in $g_T$ increases the duration of the first ISI at a rate greater than that of the second ISI. At large values of $g_T$, this leads to the first ISI being greater than the second ISI (i.e., the frequency increases with time, or acceleration). Where the 2 curves meet, the first ISI is equal to the second ISI and there is no change in frequency over time. This constant firing rate is also a feature seen in neonatal HMs (Viana et al. 1995). Because blocking the N current can eliminate the mAHP (Viana et al. 1993b), we also varied the density of the N current and measured its affects on the first 2 ISIs. The results of varying $g_N$ are similar to those of $g_T$ (Fig. 8B).

Another model experiment was performed to elucidate whether the change in density of $I_T$ affects the firing rate specifically through its influence on $I_{SK}$ by the calcium equation (see METHODS). For Fig. 8C, the calcium equation was modified to exclude the contribution from $I_T$ ($I_{Ca} = I_0 + I_{SK}$), and $g_T$ was again varied while measuring the first 2 ISIs. When $I_T$ is removed from the calcium equation, the first ISI is always smaller than the second ISI (adaptation), regardless of the value of $g_T$. In fact, increasing $g_T$ has almost no effect on the initial firing rate until $g_T$ reaches 0.16 μS. Here, the increased depolarization arising from $I_T$ allows the sodium current to initiate a second spike immediately after the repolarization of the first spike (the second spike proceeds from the ADP of the first spike). This same model experiment was performed for the $N$ current ($I_{Na} = I_0 + I_T$; Fig. 8D). Again, adaptation was seen for all values.

mAHP DURATION AND THE H CURRENT. Along with changes in firing properties, other changes accompany the HMs transition from neonatal to adulthood. These changes include a decrease in the duration of the mAHP and a large increase in the density of $I_H$ (Bayliss et al. 1994; Haddad et al. 1990; Viana et al. 1994). Bayliss et al. (1994) showed that the density of $H$ channels increases by a factor of 10 from neonatal rats to adult rats. Therefore we increased $g_H$ and measured its effects on the model. The most significant effect was seen on the duration of the mAHP (measured from the repolarizing phase of the action potential to the point in the AHP that reaches the resting potential). Neonatal HMs have a mAHP duration of 110 ms, and adult HMs have a mAHP duration of 75 ms (Viana et al. 1994). Figure 9 is a plot of mAHP duration versus $g_H$. The original model is of a neonatal HM and consequently has a mAHP duration of 109 ms. Increasing $g_H$ by a factor of 10 shortens the mAHP duration to 77 ms in the model, which is

![Fig. 7](http://jn.physiology.org/ by 10.220.32.247 on November 7, 2016)
very similar to what is seen in the data (Viana et al. 1994). This model experiment indicates that the change in the density of the H current in the data can potentially completely account for the change in mAHP duration in the data. Thus our model supports this contribution of the H current suggested by Bayliss et al. (1994).

INPUT RESISTANCE AND AP DURATION. Other changes that occur from neonatal to adulthood are a decrease in RN and a decrease in action potential duration (Viana et al. 1994). It has been suggested that the increase in H current density may be enough to account for the decrease seen in RN (Bayliss et al. 1994). However, in the model, a 10-fold increase in gH decreases RN by only 5 MΩ. The model requires a simultaneous increase of the maximum conductance of other currents active at rest, such as the A and K currents, to obtain the 20 MΩ decrease in RN seen in the data (Viana et al. 1994). Increasing these maximum conductances also causes a shortening of the action potential duration. With the nominal parameters for the neonatal HM model, the action potential duration is 1.4 ms and RN is 40 MΩ. After the 10-fold increase in H current density and a 100% increase in the maximum conductances of other currents active at rest, such as the A and K currents, the action potential duration is 1.0 ms and RN is 20 MΩ. The 0.4 ms difference in AP duration and the 20 MΩ difference in RN between neonatal and adult are consistent with experimental findings in HMs (Viana et al. 1994).

DISCUSSION

Powers (1993) developed a variable-threshold model of a cat alpha-motoneuron that was able to account for several motoneuron behaviors. That model is a simplified motoneuron model and is well suited for large networks. Powers et al. (1999) also developed other simplified motoneuron models to explore the 3 phases of adaptation seen in adult HMs (Sawczuk et al. 1995). Our model is more complex and is derived mostly from data taken from neonatal HMs. The model is able to reproduce the fine details of a HM action potential including the fAHP, the ADP, and the mAHP. The model reproduces both types of firing behavior observed in neonatal HMs: adaptation and acceleration. We proposed possible explanations for the age-dependent differences in firing properties, mAHP duration, resting RN, and action potential duration.

Apamin simulation

The model faithfully reproduces the response to a brief pulse of current when the SK current is removed. As in the experimental results, the passive properties and the depolarizing/repolarizing phase of the action potential are unchanged. This is accompanied by an enhancement of the ADP and the absence of the mAHP. Here, the critical role of the SK current in generating the mAHP can be clearly seen. The importance of ISK for repetitive firing is revealed when the brief pulse of current is lengthened to a longer step of current. Reducing gSK from 0.3 to 0.03 μS leads to a large increase in firing frequency.
accompanied with spike frequency adaptation. A similar response is obtained by reducing the total calcium current in the model to zero (not shown). Under either of these conditions, the HM model is able to display calcium-independent spike frequency adaptation, which has been shown to occur experimentally (Lape and Nistri 2000; Sawczuk et al. 1997; Viana et al. 1993b). In the model, this adaptation is caused by the slow inactivation of $I_{\text{NaP}}$. However, under normal conditions, calcium and calcium-sensitive currents dominate the firing behavior of the model.

For the simulation in Fig. 4B, $g_{\text{SK}}$ was reduced to 0.03 $\mu$S and not 0 $\mu$S. When $g_{\text{SK}}$ is completely removed ($g_{\text{SK}} = 0$), the model does not display the initial spike frequency adaptation. Instead, the model displays acceleration caused by inactivation of the A current. This contradicts the role of the A current suggested by Lape and Nistri (1999) where they propose that the A current causes adaptation in HMs. The acceleration occurs regardless of whether the model parameters (before reduction of $g_{\text{SK}}$ attributed to apamin) are set to produce adaptation, as in Fig. 6, or acceleration, as in Fig. 7. As discussed in MODEL DEVELOPMENT, our implementation of the A current is simpler than the description given by Lape and Nistri (1999). Alternatively, a multicompart model similar to that of Booth and Rinzel (1995) that physically separates the fast and slow currents may provide a more robust response.

Age-dependent changes

The model provides a possible explanation for the change in repetitive firing properties observed during development. If $I_T$ or $I_N$ is included in the calcium equation, then increasing the density of that current in the model changes the firing properties from adaptation to acceleration (Fig. 8, A and B). When the current density is low, the SK current is only partially activated during the first spike. The level of internal calcium increases during the second spike and causes summation of the SK current, which produces adaptation. When the T or N current density is large, the SK current is almost completely activated during the first spike. Because both the T and N currents quickly inactivate after the first spike, much less calcium enters the cell through those channels during subsequent spikes. This causes the level of internal calcium to reach its maximum during the first spike. As the level of internal calcium decreases, the activation of the SK current also decreases. The smaller SK current allows subsequent spikes to occur sooner (i.e., acceleration). This phenomenon is dynamic, and not merely a by-product of increasing the overall level of calcium entering the cell. If the density of the P current (a slowly activating and noninactivating calcium current) is increased, the model continues to show adaptation.

Experiments show that the density of the current resistant to the 3 HVA current antagonists (considered to be the T current) decreases during postnatal development. The decrease seen experimentally in T current density is measured between days 3 and 4 postnatal (Umemiya and Berger 1994). Experiments have shown that the majority of HMs show spike frequency adaptation during the 1st wk postnatal, and acceleration during the 2nd wk (Viana et al. 1995). Reducing the T current in the model changes the firing characteristics from acceleration to adaptation. If experiments confirm that the T current continues to monotonically decrease as the rat ages, then the model’s results do not correlate (until the adult age). If a change in T current density as modeled is the sole cause of the change in repetitive firing properties during aging, then the T current density will have to increase during the 2nd wk postnatal and then decrease before adulthood. There have been no reports of an age-dependent change in N current density in HMs.

Figure 8 demonstrates that varying the density of a calcium current only affects the firing properties (adaptation vs. acceleration) if that calcium current has an influence on the SK current. Therefore colocalization of calcium channels and the SK channels could play a role in determining HM firing behavior. Colocalization between specific calcium channels and calcium-dependent potassium channels has been observed in other neurons (Bowden et al. 2001; Kobayashi et al. 1997; Marrion and Tavalin 1998) and was proposed in HMs by Viana et al. (1993b). Colocalization would allow the influx of calcium from only certain channel types to affect activation of the SK current. Thus a current such as the L-type calcium current (not modeled) that makes up <10% of the total calcium current in HMs could have a substantial effect on the shape of the action potential. The dynamics of a single calcium channel type could control the firing properties of HMs through the SK current. If the SK channels are localized to the P or L channels at birth, and then shift their localization to the T or N channels, this will change the firing properties from adaptation to acceleration. Another possibility is that the SK channels may change from a global channel to one that is localized to a certain calcium channel type during aging, again changing the firing properties with age. To verify this, experiments to determine the existence of colocalization between the SK current and any of the calcium currents in HMs would need to be performed. Viana et al. (1993a) show a neuron where pharmacologically blocking N channels removes the mAHP. Although it is not stated, this neuron appears to display acceleration. This could then be tested by blocking P and/or L channels in a HM that displays adaptation.

Mammalian motoneurons experience anatomical and physiological changes during development (reviewed in Cameron and Nunez-Abades 2000). Some of the age-dependent changes of neonatal rat HMs (reviewed in Berger et al. 1996) have been explored here. These changes include a decrease in mAHP duration, a decrease in $R_N$, a decrease in action potential duration, a change in repetitive firing properties, and changes in the densities of different ion channels. Factors that affect the duration of the mAHP in the model include 1) the magnitude and time course of the calcium currents, 2) the SK current, 3) the A current, 4) the H current, and 5) the depletion rate of calcium from the cell. We showed in Fig. 9 that, in the model, the 10-fold increase in density of the H current is enough to account for the decrease in mAHP duration observed between neonatal and adult rats. However, the change in H current density is not enough to account for all of the age-dependent changes seen in HMs. According to Viana et al. (1994), the resting membrane potential of HMs is the same for neonatal and adult rats. In the model, the resting membrane potential increases by about 5 mV as the H current density is increased. Also, increasing the density of the H current in the model cannot completely account for the decrease in $R_N$ or produce the decrease in action potential duration observed in HMs (Viana et al. 1994). Concurrent changes in other currents are needed to account for these behaviors in the model. Cameron
and Nunez-Abades (2000) agree that the decrease in $R_N$ is partly caused by a proliferation of ion channels. However, they suggest that this decrease is also caused by an increase in the number of synaptic inputs, something that is not included in our model.

Adult HMs fire at a higher rate than neonatal HMs (Viana et al. 1995). The decrease in mAHP duration could allow for the higher firing rates observed in adult HMs. In addition to its possible role in modulating the steady-state firing rate, the H current could potentially play a role in spike frequency adaptation seen in adult HMs. In the model, the magnitude of the H current between spikes (during the mAHP) decreases over time with a time constant of about 300 ms during a train of action potentials. If the density of the H current is sufficiently large, this spike-to-spike decrease in H current magnitude could result in an increase in mAHP duration between spikes. However, increasing $g_H$ to 0.1 $\mu$S causes only a small amount of adaptation in the model (<1 Hz decrease over 1 s).

**Limitations of the model**

**CURRENTS.** The currents included in this model are only those known to exist in neonatal rat HMs (reviewed in Berger 2000). We did not include those currents that have been observed in other motoneurons or other animal species. Currents such as the voltage- and calcium-activated potassium current (BK) and the sodium-activated potassium current (K, NA) found in other motoneurons (reviewed in McLarnon 1995; Rekling et al. 2000) could also exist in neonatal rat HMs. These currents could certainly affect the model’s behavior, but are not included because there are no data describing these currents in neonatal HMs. This is both a strength and a limitation of the model. A potential role of the BK current is the age- and calcium-dependent change in action potential shape during a train of action potentials observed in HMs (Viana et al. 1995). An increase in density of a BK current, or an increase in density of a calcium current that activates a BK current, could cause this age-dependent change.

**MORPHOLOGY.** Many motoneuron morphologies have been described previously. Some of these studies include a 3-dimensional analysis of cat alpha-motoneuron morphology (Cullheim et al. 1987a,b) and phrenic motoneuron morphology from a neonatal rat (Lindsay et al. 1991). HM morphologies have also been investigated in the cat (Withington-Wray et al. 1988), guinea-pig (Mosfeldt Laursen and Rekling 1989; Viana et al. 1990), and rat (Altschuler et al. 1994). The morphology of rat genioglossal motoneurons has been shown to change during postnatal development (Mazza et al. 1992; Nunez-Abades and Cameron 1995; Nunez-Abades et al. 1994). It is clear that morphology plays an important functional role in motoneurons (reviewed in Rekling et al. 2000). However, our single-compartment HM model is able to accurately reproduce several electrophysiological characteristics of HMs. Extension of this model will be required to explain the effects of synaptic integration, including modeling dendritic structures. Developmental changes in HM morphology may also partially account for the age-dependent changes in resting membrane potential and $R_N$.

**APPENDIX: MODEL EQUATIONS**

**Sodium current: $I_{Na}$**

$$I_{Na} = g_{Na} m_h h(V - E_{Na})$$

$$m_h(V) = \frac{1}{1 + e^{-(V + 30.6)/8.5}} \quad \tau_{m}(V) = 0.1$$

$$h(V) = \frac{1}{1 + e^{-(V + 44.1)/11.7}} \quad \tau_{h}(V) = 3.5 \frac{e^{V + 33.04} + e^{-V + 33.04}}{e^{V + 33.04} + e^{-V + 33.04} + 1}$$

**Persistent sodium current: $I_{NaP}$**

$$I_{NaP} = g_{NaP} m_{NaP} h_{NaP}(V - E_{Na})$$

$$m_{NaP}(V) = \frac{1}{1 + e^{-(V + 42.7)/14.1}} \quad \tau_{m}(V) = 0.1$$

$$h_{NaP}(V) = \frac{1}{1 + e^{V + 45.07}} \quad \tau_{h}(V) = 150$$

**Delayed-rectifier current: $I_K$**

$$I_K = g_{K} h^4(V - E_K)$$

$$n_(V) = \frac{1}{1 + e^{V + 30.255}} \quad \tau_{n}(V) = \frac{2.5}{e^{V + 30.255} + e^{-V + 30.255} + 0.01}$$

**Leak current: $I_{leak}$**

$$I_{leak} = g_{leak}(V - E_{leak})$$

**LVA calcium current: $I_T$**

$$I_T = g_{T} m_h h(V - E_{Ca})$$

$$m_h(V) = \frac{1}{1 + e^{-(V + 30.6)/8.5}} \quad \tau_{m}(V) = \frac{5}{e^{V + 28.05} + e^{-V + 28.05} + 2}$$

$$h(V) = \frac{1}{1 + e^{V + 33.07}} \quad \tau_{h}(V) = \frac{20}{e^{V + 30.05} + e^{-V + 30.05} + 1}$$

**HVA calcium current: $I_N$**

$$I_N = g_{N} m_h h(V - E_{Ca})$$

$$m_h(V) = \frac{1}{1 + e^{V + 30.65}} \quad \tau_{m}(V) = 5$$

$$h(V) = \frac{1}{1 + e^{V + 33.07}} \quad \tau_{h}(V) = 25$$

**HVA calcium current: $I_P$**

$$I_P = g_{P} m_p h(V - E_{Ca})$$

$$m_p(V) = \frac{1}{1 + e^{V + 33.07}} \quad \tau_{m}(V) = 10$$

**Calcium-dependent potassium current: $I_{SK}$**

$$I_{SK} = g_{SK} z_{SK} (V - E_{K})$$

$$z_{SK}([Ca^{2+}]) = \frac{1}{1 + \left(\frac{0.003}{[Ca^{2+}]}\right)^{0.5}} \quad \tau_{SK}([Ca^{2+}]) = 1$$
Fast-transient potassium current: $I_A$

$$I_A = \tilde{g}_m h_A (V - E_k)$$

$$m_{h_A}(V) = \frac{1}{1 + e^{(V+35)/9}}$$

$$h_{h_A}(V) = \frac{1}{1 + e^{(V+70)/7.4}}$$

Hyperpolarization-activated current: $I_H$

$$I_h = \tilde{g}_m h_h (V - E_k)$$

$$m_{h_h}(V) = \frac{1}{1 + e^{(V+30)/-15}}$$

$$h_{h_h}(V) = \frac{1}{1 + e^{(V+74)/7.6}}$$

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