Differential contributions of somatic and dendritic calcium-dependent potassium currents to the control of motoneuron excitability following spinal cord injury

Sharmila Venugopal · Thomas M. Hamm ·
Ranu Jung

Received: 26 July 2011 / Revised: 4 December 2011 / Accepted: 23 January 2012 / Published online: 17 February 2012
© Springer Science+Business Media B.V. 2012

Abstract The hyperexcitability of alpha-motoneurons and accompanying spasticity following spinal cord injury (SCI) have been attributed to enhanced persistent inward currents (PICs), including L-type calcium and persistent sodium currents. Factors controlling PICs may offer new therapies for managing spasticity. Such factors include calcium-activated potassium (KCa) currents, comprising in motoneurons an after-hyperpolarization-producing current (I_{KCaN}) activated by N/P-type calcium currents, and a second current (I_{KCaL}) activated by L-type calcium currents (Li and Bennett in *J neurophysiol* 97:767–783, 2007). We hypothesize that these two currents offer differential control of PICs and motoneuron excitability based on their probable somatic and dendritic locations, respectively. We reproduced SCI-induced PIC enhancement in a two-compartment motoneuron model that resulted in persistent dendritic plateau potentials. Removing dendritic I_{KCaL} eliminated primary frequency range discharge and produced an abrupt transition into tertiary range firing without significant changes in the overall frequency gain. However,

I_{KCaN} removal mainly increased the gain. Steady-state analyses of dendritic membrane potential showed that I_{KCaL} limits plateau potential magnitude and strongly modulates the somatic injected current thresholds for plateau onset and offset. In contrast, I_{KCaN} had no effect on the plateau magnitude and thresholds. These results suggest that impaired function of I_{KCaL} may be an important intrinsic mechanism underlying PIC-induced motoneuron hyperexcitability following SCI.

Keywords Motoneuron model · Persistent inward currents · Spinal cord injury · Steady-state analysis · Calcium currents · Calcium dependent potassium currents · Dendritic currents · Hyperexcitability · Spasticity

Introduction

Reflexes are involuntary motor behaviors triggered by various sensory stimuli (e.g., tendon tap reflex), often integrated into centrally generated movements so as to contribute to the control and coordination of those movements. Chronic spinal cord injury (SCI) often results in exaggerated reflexes (hyperreflexia) below the level of injury, producing unwanted movements and impaired control of voluntary movements. Hyperreflexia and other concomitant changes such as increased muscle tone lead to spasticity (Lance 1980). Neural mechanisms implicated in hyperreflexia include enhancement of synaptic excitation (Baker and Chandler 1987), down-regulation of inhibition (Shapiro 1997) and probably increased excitability of interneurons mediating the spinal reflexes (ElBasiouny et al. 2010). In particular, spinal alpha-motoneurons that form the final common neural pathway driving the skeletal musculature become hyperexcitable (Bennett et al. 2001b).

S. Venugopal
Department of Integrative Biology & Physiology,
University of California Los Angeles, Los Angeles,
CA 90095, USA

T. M. Hamm
Division of Neurobiology, Barrow Neurological Institute,
St. Joseph's Hospital, Phoenix, AZ 85013, USA

R. Jung (✉)
Department of Biomedical Engineering, Florida
International University, EC 2602, 10555 W Flagler Street,
Miami, FL 33174, USA
e-mail: rjung@fiu.edu

The observed hyperexcitability has been attributed to an enhancement of intrinsic persistent inward currents (Bennett et al. 2001a; Li et al. 2004a). Persistent inward currents (PICs) include a low-voltage activated L-type calcium current (I_{CaL}) and a sub-threshold persistent sodium (I_{NaP}) current. Under normal conditions, PICs are activated by brain-derived serotonin (5-HT) and other monoamines (Hounsgaard et al. 1988; Heckman et al. 2005). Functionally, PICs amplify synaptic inputs and influence the dynamic transformation of such inputs into motoneuron frequency code that directly translates into muscle force. Therefore, PICs are important determinants of motoneuron excitability. Enhancement of PICs following SCI can result in spasms (Gorassini et al. 2004; Harvey et al. 2006b). Hence regulation of PICs by intrinsic as well as synaptic mechanisms is potentially a critical factor in the management of motoneuron excitability following SCI. We recently showed that PIC regulation can be restored by increasing the strength and kinetics of dendritic inhibition (Venugopal et al. 2009a, b, 2011). Here we study an alternative mechanism involving intrinsic calcium-dependent potassium currents in the control of PICs and motoneuron excitability.

Small-conductance calcium-activated potassium (SK) currents are present in many types of neurons. They are important for the production of after-hyperpolarization (AHP) following an action potential and hence in the control of membrane excitability. In motoneurons, N-type calcium-dependent SK currents are involved in the production of medium AHP or mAHP (lasting a few tens of seconds) (Li and Bennett 2007). The mAHP has been suggested to limit the gain (slope of the injected current—firing frequency or I–f curves) and inter-spike interval variability (Manuel et al. 2005, 2006). It is also suggested to be important for grading PICs (Elbasiouny et al. 2006). Recently, a L-type calcium-dependent (calcium PIC-dependent) SK current that was not involved in mAHP production (i.e., was insensitive to N-type calcium channel blockers) was reported by Li and Bennett (2007). Interestingly, following SCI, mAHP remained unchanged whereas a reduction in calcium-PIC dependent SK current relative to L-type calcium current was noted, suggesting that changes in this potassium current may be a factor in the increased L-type calcium current and hyperexcitability following SCI. However the selective roles for these two populations of N- and L-type calcium-dependent SK currents in PIC control remain unclear. In the present study, we use a computational model for a SCI motoneuron to examine the selective roles of the two SK currents on motoneuron excitability. In particular, we hypothesized that the L-type calcium-dependent potassium current has a greater role in the control of PIC-mediated motoneuron hyper-excitability that may underlie spasticity resulting

from chronic SCI. Short form of this work has been published as an abstract and presented as a poster (Venugopal et al. 2010).

Methods

We utilize our recently developed conductance-based SCI motoneuron model (Venugopal et al. 2009a, b, 2011) to examine the contribution of SK currents to PIC regulation. Briefly, the model consists of two electrotonically coupled compartments: soma (or cell body) and dendrite. Figure 1 shows the schematic of the model showing the ionic currents included in each compartment. The soma includes the action potential generating currents including the fast sodium (I_{Na}), delayed rectifier type potassium (I_{Kd}), medium after-hyperpolarization generating N-type calcium (I_{CaN}) and N-type calcium-dependent potassium (I_{KCaN}) along with a leak current (I_{Leak}). The dendritic compartment consists of the PICs: low-threshold L-type calcium (I_{CaL}) and persistent sodium (I_{NaP}) currents as well as the I_{CaL} dependent potassium current (I_{KCaL}) and a leak current. Thus in this model, the selective dependence of SK channels on N-type and L-type calcium channels is presumed to depend on colocalization with N-type and L-type calcium channels that are located separately in soma and dendrites (see “Discussion”).

Following chronic SCI, intrinsic hyperexcitability of the motoneuron is evidenced by the presence of a counter-clockwise hysteresis in the I–f relationship and persistent

Fig. 1 Schematic of the two-compartment SCI motoneuron model showing distribution of the intrinsic ionic currents. The currents included in the model are as follows: spike generating—fast sodium (I_{Na}) and delayed rectifier-type potassium (I_{Kd}); medium after-hyperpolarization (mAHP) generating—N-type calcium (I_{CaN}) and N-type calcium activated potassium (I_{KCaN}); leak potassium currents essential for stable resting potential (I_{Leak}); persistent inward currents—L-type calcium (I_{L-Ca}) and persistent sodium (I_{NaP}); sustained potassium—L-type calcium activated potassium (I_{KCaL}); Soma injected current— I_{SOMA} ; soma and dendrite coupling current (I_{coup})

plateau activation (Bennett et al. 2001b). These properties were not observed after acute spinal transection. We reproduce the firing properties including I–f hysteresis and persistent plateau activation in the SCI motoneuron model (also see (Venugopal et al. 2009a, b, 2011)). In order to do so, we assume values of conductances for the PICs, I_{CaL} and I_{NaP} that generate a counter-clockwise I–f hysteresis (Li and Bennett 2003; Li et al. 2004a) such that the ratio of the I_{CaL} and I_{NaP} conductances are ~ 1.772 as noted in (Li et al. 2004a). Furthermore, we set the dendritic I_{KCaL} to be a smaller fraction of I_{CaL} as observed after chronic SCI (Li and Bennett 2007) (see (Venugopal et al. 2011) and Discussion for further model considerations).

The somatic and dendritic membrane potentials follow the standard Hodgkin-Huxley formalism with current balance equations as follows:

$$C_m \frac{dV_{SOMA}}{dt} = -I_{Leak} - I_{Na} - I_{Kdr} - I_{CaN} - I_{KCaN} - I_{Coup} + I_{SOMA}$$

$$C_m \frac{dV_{DENDRITE}}{dt} = -I_{Leak} - I_{CaL} - I_{NaP} - I_{SK-L} - I_{Coup}$$

where, $C_m = 1 \mu F/cm^2$ as for most cells.

The ion channel gating kinetics of motoneuron soma are given by the following equations:

$$\frac{dh_{Na}}{dt} = \frac{h_{Na\infty}(V_{SOMA}) - h_{Na}}{\tau_{Na}(V_{SOMA})};$$

$$\tau_{Na}(V_{SOMA}) = \frac{30}{\left(e^{\frac{(V_{SOMA}-(-50))}{15}} + e^{\frac{(V_{SOMA}-(-50))}{16}} \right)}$$

$$\frac{dn_{Kdr}}{dt} = \frac{n_{Kdr\infty}(V_{SOMA}) - n_{Kdr}}{\tau_{Kdr}(V_{SOMA})};$$

$$\tau_{Kdr}(V_{SOMA}) = \frac{7}{\left(e^{\frac{(V_{SOMA}-(-40))}{40}} + e^{\frac{(V_{SOMA}-(-40))}{50}} \right)}$$

$$\frac{dm_{CaN}}{dt} = \frac{m_{CaN\infty}(V_{SOMA}) - m_{CaN}}{\tau_{CaN}^m};$$

$$\frac{dh_{CaN}}{dt} = \frac{h_{CaN\infty}(V_{SOMA}) - h_{CaN}}{\tau_{CaN}^h};$$

where $\tau_{CaN}^m = 4$ ms, $\tau_{CaN}^h = 40$ ms are the activation and inactivation time constants respectively for N-type calcium currents similar to (Booth et al. 1997).

The dendritic ion channel gating kinetics follow:

$$\frac{dm_{CaL}}{dt} = \frac{m_{CaL\infty}(V_{DENDRITE}) - m_{CaL}}{\tau_{CaL}^m};$$

$$\frac{dm_{NaP}}{dt} = \frac{m_{NaP}(V_{DENDRITE}) - m_{NaP}}{\tau_{NaP}^m};$$

$$\frac{dh_{NaP}}{dt} = \frac{h_{NaP\infty}(V_{DENDRITE}) - h_{NaP}}{\tau_{NaP}^h};$$

where the time constant of activation for L-type calcium current is, $\tau_{CaL}^m = 40$ ms (Booth et al. 1997). The activation time constant of persistent sodium currents was chosen similar to L-type calcium, $\tau_{NaP}^m = 40$ ms, while the inactivation was in the order of magnitude as in other mammalian neurons with $\tau_{NaP}^h = 1,000$ ms.

The intracellular calcium concentration in the somatic compartment is modeled to depend on the high-voltage activated CaN calcium currents while the calcium concentration in the dendritic compartment is modeled to depend explicitly on L-type Ca^{2+} PIC as follows:

$$\frac{dCa_{SOMA}}{dt} = f(\alpha I_{CaN} - K_{Ca} Ca_{SOMA})$$

$$\frac{dCa_{DENDRITE}}{dt} = f(\alpha I_{CaL} - K_{Ca} Ca_{DENDRITE})$$

where the constant $f = 0.01$ is the percent of free to bound calcium, $\alpha = 0.009$ mol/C/ μm converts the total calcium current in each compartment to Ca^{2+} concentration and $K_{Ca} = 2$ ms $^{-1}$ is the calcium removal rate assumed to be similar in both compartments (also see (Booth et al. 1997)).

Somatic currents are:

$$I_{Leak} = g_L(V_{SOMA} - V_K)$$

$$I_{Na} = g_{Na}(m_{Na\infty}(V_{SOMA}))^3 h_{Na}(V_{SOMA} - V_{Na})$$

$$I_{Kdr} = g_{Kdr}(n_{Kdr})^4 (V_{SOMA} - V_{Kdr})$$

$$I_{CaN} = g_{CaN}(m_{CaN})^2 h_{CaN}(V_{SOMA} - V_{Ca})$$

$$I_{KCaN} = g_{KCaN} \frac{Ca_{SOMA}}{0.2 + Ca_{SOMA}}$$

$$I_{Coup} = g_{coup} \frac{(V_{DENDRITE} - V_{SOMA})}{p_{area}}$$

where the maximal ionic conductances are (in mS/cm 2): $g_L = 0.51$; $g_{Na} = 80$; $g_{Kdr} = 100$; $g_{CaN} = 14$; $g_{KCaN} = 6$. The ratio of somatic area to the total surface area of the motoneurons $p_{area} = 0.1$ and the soma-dendrite coupling conductance $g_{coup} = 0.1$ mS/cm 2 .

The dendritic ionic currents are:

$$I_{Leak} = g_L(V_{DENDRITE} - V_K)$$

$$I_{CaL} = g_{CaL} m_{CaL}(V_{DENDRITE} - V_{Ca})$$

$$I_{NaP} = g_{NaP} m_{NaP} h_{NaP}(V_{DENDRITE} - V_{Na})$$

$$I_{SK-L} = g_{SK-L} \frac{Ca_{DENDRITE}}{0.2 + Ca_{DENDRITE}}$$

$$I_{Coup} = g_{coup} \frac{(V_{SOMA} - V_{DENDRITE})}{1 - p_{area}}$$

where the maximal ionic conductance are (in mS/cm 2): $g_{CaL} = 0.27$; $g_{NaP} = 0.13$; $g_{SK-L} = 1$ and the ionic reversal potentials are (in mV): $V_K = -80$, $V_{Ca} = +80$, $V_{Na} = 55$.

Soma steady-state voltage-dependent channel gating functions are:

$$m_{Na\infty}(V_{SOMA}) = \frac{1}{1 + e^{\left(\frac{V_{SOMA} - \theta_{mNa}}{K_{mNa}}\right)}}$$

$$h_{Na\infty}(V_{SOMA}) = \frac{1}{1 + e^{\left(\frac{V_{SOMA} - \theta_{hNa}}{K_{hNa}}\right)}}$$

$$\tau_{Na}(V_{SOMA}) = \frac{30}{e^{\left(\frac{V_{SOMA} - (-50)}{15}\right)} + e^{\left(\frac{V_{SOMA} - (-50)}{16}\right)}}$$

$$n_{Kdr\infty}(V_{SOMA}) = \frac{1}{1 + e^{\left(\frac{V_{SOMA} - \theta_{nKdr}}{K_{nKdr}}\right)}}$$

$$\tau_{Kdr}(V_{SOMA}) = \frac{7}{e^{\left(\frac{V_{SOMA} - (-40)}{40}\right)} + e^{\left(\frac{V_{SOMA} - (-40)}{50}\right)}}$$

$$m_{CaN\infty}(V_{SOMA}) = \frac{1}{1 + e^{\left(\frac{V_{SOMA} - \theta_{mCaN}}{K_{mCaN}}\right)}}$$

$$h_{CaN\infty}(V_{SOMA}) = \frac{1}{1 + e^{\left(\frac{V_{SOMA} - \theta_{hCaN}}{K_{hCaN}}\right)}}$$

where the half-activation and inactivation voltages for the somatic channel gating functions are (in mV) $\theta_{mNa} = -35$, $\theta_{hNa} = -55$, $\theta_{nKdr} = -28$, $\theta_{mCaN} = -30$, $\theta_{hCaN} = -45$. The voltage sensitivities of activation and inactivation are (in mV) $K_{mNa} = 7.8$, $K_{hNa} = 7$, $K_{nKdr} = 12$, $K_{mCaN} = 5$, $K_{hCaN} = 5$.

Dendritic steady-state voltage-dependent channel activation/inactivation functions are:

$$m_{CaL\infty}(V_{DENDRITE}) = \frac{1}{1 + e^{\left(\frac{V_{DENDRITE} - \theta_{mCaL}}{K_{mCaL}}\right)}}$$

$$m_{NaP}(V_{DENDRITE}) = \frac{1}{1 + e^{\left(\frac{V_{DENDRITE} - \theta_{mNaP}}{K_{mNaP}}\right)}}$$

$$h_{NaP\infty}(V_{DENDRITE}) = \frac{1}{1 + e^{\left(\frac{V_{DENDRITE} - \theta_{hNaP}}{K_{hNaP}}\right)}}$$

where the half-activation and inactivation voltages for the dendritic channel gating functions are (in mV) $\theta_{mCaL} = -39$, $\theta_{mNaP} = -48$, $\theta_{hNaP} = -35$ and the voltage sensitivities of gating functions are (in mV) $K_{mCaL} = 7$, $K_{mNaP} = 3$, $K_{hNaP} = 6$.

Simulations and steady-state analyses were performed using XPPAUT (Doedel 1981; Ermentrout 2002) and MATLABTM was utilized to make the figures. One and two-parameter bifurcation was used to demonstrate the changes in the steady-state responses with respect to changes in one or more parameters including somatic injected current, somatic and dendritic KCa conductances.

Results

Motoneurons develop exaggerated PICs chronically after spinal cord injury (SCI) (Li and Bennett 2003; Li et al. 2004a). The PICs, thought to be of dendritic origin, produce a counter-clockwise hysteresis in the I–f relationship during a somatic current injection (Bennett et al. 2001a). We reproduce these firing properties in the model motoneuron as illustrated in Fig. 2a. The response of the somatic (V_{SOMA}) and dendritic ($V_{DENDRITE}$) voltages to a triangular ramp current injection in the soma (I_{SOMA}) is shown. As I_{SOMA} ramps up (bottom trace in Fig. 2a), $V_{DENDRITE}$ slowly develops a plateau potential marking the activation of PICs. The dendritic plateau provides a strong

Fig. 2 Firing properties of the SCI motoneuron model. **a** Voltage and frequency response to a triangular ramp current injection (bottom trace) increasing from 0 to 20 nA (up ramp) and then down to −10 nA (down ramp) at the rate of 5 nA/s. The dendritic voltage $V_{DENDRITE}$ develops a plateau during the up ramp; a concomitant acceleration in somatic spikes and a jump up in the firing frequency are noted. The time axes are the same for each panel and that for the current ramp. **b** The I–f relationship showing counter-clockwise hysteresis. Three frequency ranges are noted: 1^0 or primary range with lower firing frequencies before the plateau onset, 2^0 or secondary range during the plateau development, 3^0 range during sustained plateau activation

depolarization at the soma resulting in higher (plateau-mediated) firing frequencies. The resultant I–f curve is shown in Fig. 2b. The 1^0 frequencies mark the primary range before plateau activation, the 2^0 frequencies mark the secondary range during plateau activation and the 3^0 frequencies correspond to the tertiary range of motoneuron firing.

Model reproduces effects of pharmacological blockade of small-conductance KCa currents

Experimental reports have shown that apamin, a non-specific SK channel blocker, induces intense firing in SCI motoneurons that in some cases resulted in virtually no termination of firing even with injection of very large hyperpolarizing (negative) currents at the soma (Li and Bennett 2007). This apamin-induced uncontrollable firing is partly mediated by its action on the mAHP conductance. Hence, we simulate the apamin effect in our model by eliminating both somatic and dendritic KCa conductances ($g_{KCaN}^{\text{somatic}} = 0$, $g_{KCaL}^{\text{dendritic}} = 0$) that in turn also eliminated the mAHP (see Fig. 3a, b). In these and in subsequent figures, “control” refers to the condition with default values of the two KCa conductances that result in firing properties similar to a SCI motoneuron as that shown in Fig. 2. Though elimination of mAHP increased high-frequency discharge, it did not eliminate the slow firing often observed during the last few spikes of the decreasing ramp. Such slow firing is attributed to NaP currents that cause slow sub-threshold ramping of the membrane potential (double arrows in Fig. 3b) eventually resulting in a spike (Li and Bennett 2007). Experiments have further shown that elimination of N-type calcium currents by Conotoxin MVIIC abolishes mAHP while elimination of L-type calcium currents by Nimodipine did not abolish the mAHP (Li and Bennett 2007). We verify this result in our model by selectively setting $g_{KCaN}^{\text{somatic}}$ and $g_{KCaL}^{\text{dendritic}}$ to zero (see Fig. 3c). In these simulations, we note that the classic fAHP or fast AHP due to activation of delayed rectifier potassium currents are not affected as also observed in (Li and Bennett 2007). *In summary, the model reproduces experimental observation that mAHP is mediated by N-type and not L-type KCa currents.*

Differential role of somatic and dendritic KCa currents in the control of motoneuron discharge

The mAHP has been suggested to play an important role in the control of motoneuron gain (Manuel et al. 2005, 2006). However, whether mAHP influences PIC-mediated motoneuron discharge is unknown. Moreover, the role of the

dendritic L-type calcium-dependent KCa in the control of PIC activation and deactivation is yet speculative. Indeed, whether enhancement of PICs following SCI can partly result from a down-regulation of calcium-dependent potassium currents colocalized with L-type calcium currents in dendrites is unclear. We addressed these questions by examining the motoneuron firing properties upon selective removal of the mAHP-causing $g_{KCaN}^{\text{somatic}}$ and the L-type calcium-dependent $g_{KCaL}^{\text{dendritic}}$ conductances. Figure 4b shows the I–f response to the triangular current ramp as in Fig. 2a upon removal of the somatic KCa conductance ($g_{KCaN}^{\text{somatic}} = 0$). Note that in comparison with the control response in Fig. 4a, elimination of $g_{KCaN}^{\text{somatic}}$ greatly increased the firing frequency in all the three ranges. This increase in frequency gain is consistent with the inverse relationship observed between mAHP size and slope of I–f relationship by (Manuel et al. 2005, 2006). We further observed a nominal reduction in the input threshold for recruitment (i.e., firing onset) in the primary range and a larger reduction in threshold for PIC onset in the secondary range. Alternatively, elimination of dendritic KCa did not significantly alter overall firing frequencies of the motoneuron suggesting very little gain modulation (see Fig. 4c). It did, however, completely eliminate the primary range frequencies and increased the slope of secondary frequencies suggesting its greater control on PIC activation. Moreover, elimination of dendritic KCa enhanced the dendritic plateau magnitude (see Fig. 4d) while elimination of somatic KCa only advanced the plateau onset by a small amount as illustrated in the same figure. *In summary, dendritic KCa currents exerted greater control on PIC induced plateau and firing frequencies while mAHP-mediated somatic KCa currents were important for limiting the overall firing frequencies (gain control) of the motoneuron.*

Steady-state analyses of the selective role of dendritic KCa in PIC control

We next examined the selective role of somatic and dendritic KCa in controlling the input (I_{SOMA}) thresholds for PIC activation/onset and deactivation/offset using a steady-state bifurcation analysis. To do so, we generated the steady-state bifurcation diagram of V_{DENDRITE} with I_{SOMA} as the bifurcation parameter (see Fig. 5a). The theoretical onset and offset thresholds for PIC/plateau lie at the lower and upper knees of the S-shaped steady-state curves respectively (see black dots in the figure) (Booth et al. 1997; Lee and Heckman 1998a). The dendritic voltage response to the slow triangular ramp I_{SOMA} (such as in Fig. 2a) was superimposed on the resultant S-shaped steady-state curve and the arrows further illustrate the evolution of V_{DENDRITE} plateau in time. Note that when somatic KCa is blocked (Fig. 5b), the PIC onset and offset

Fig. 3 Simulated pharmacological blockade of Ca^{2+} -dependent potassium channels. **a** Traces shown include somatic injected current (I_{SOMA}), somatic voltage, V_{SOMA} (black) and dendritic voltage, $V_{DENDRITE}$ (red) and somatic firing frequencies (black circles). **Left:** Response to a triangular ramp current injection under control conditions; the ratio of the plateau-causing dendritic persistent Ca^{2+} and Na^{+} conductances was slightly adjusted from 1.77 to 1.68 to more closely mimic Fig. 2 in (Li and Bennett 2007). **Right:** The somatic and dendritic Ca^{2+} -dependent potassium conductances were set to zero ($g_{KCaN} = 0$, $g_{KCaL} = 0$) to mimic the effect of the SK channel blocker apamin in (Li and Bennett 2007). **b** The first and last spikes are expanded in time under control and apamin simulated conditions to highlight the elimination of mAHP and not fAHP. The

double arrows near the sub-threshold regions of the last spikes denote that the slow ramping of membrane potential before spike generation is characteristic of I_{NaP} and is not abolished on mAHP elimination. **c** Illustration of the effects of eliminating the low-voltage activated persistent calcium current ($g_{CaL} = 0$) and high-voltage activated N-type calcium current ($g_{CaN} = 0$) mimicking nimodipine and ω -Conotoxin application respectively. Note that removal of the persistent calcium current does not eliminate mAHP (middle trace) while removal of the N-type calcium current completely abolishes the mAHP (right trace). The left trace shows control; middle trace shows an overlay of the control (in grey) on the $g_{CaL} = 0$ trace (in red). (Color figure online)

thresholds do not change. However, we did observe a slightly higher depolarized state of dendritic plateau on the upper branch of the S-shaped curve (see near merger of the upper dashed line with solid line in Fig. 5b). This slight enhancement of the plateau is likely due to a positive feedback from the soma (as a result of the electrotonic

coupling) in the absence of the mAHP. In contrast, removal of dendritic KCa currents (Fig. 5c) produced substantial negative shifts in the I_{SOMA} thresholds for both PIC onset and offset (note the shifts in lower and upper black circles in comparison with control). Moreover, the dendritic voltage was significantly more depolarized suggesting

Fig. 4 Differential role of somatic and dendritic KCa currents. **a–c** Injected current–firing frequency curves under control (**a**), without somatic KCa ($g_{KCaN} = 0$) (**b**) and without dendritic KCa ($g_{KCaL} = 0$) (**c**) respectively. The 1^0 , 2^0 and 3^0 indicate the primary, secondary and tertiary firing frequencies respectively. **d** Development of the dendritic plateau under the three conditions of control (grey), somatic $g_{KCaN} = 0$ (black) and dendritic $g_{KCaL} = 0$ (dashed) respectively

enhancement of PIC magnitude. These results suggest that dendritic KCa is critical for controlling the magnitude and thresholds of PICs. In Fig. 6, we further utilize two-parameter bifurcation diagrams to illustrate the changes in PIC onset and offset thresholds for a wide range of somatic

Fig. 5 Steady-state analyses of the role of somatic and dendritic KCa currents in the control of dendritic PIC. **a–c** Black traces show S-shaped steady-state curves for dendritic voltage under control (**a**), no somatic KCa (**b**) and no dendritic KCa (**c**) conditions. Bold lines show stable steady states, dotted lines show unstable steady states. The lower and upper black circles denote the voltage at which dendritic PICs onset and offset respectively. The red traces show the projection of the response to slow somatic current ramp current injection as in Fig. 2a in each case. Arrows denote the development of dendritic plateau with initial condition near the black circle at the lower branch of the S-shaped curve

KCa (Fig. 6a) and dendritic KCa (Fig. 6b) conductances. Note that the I_{SOMA} thresholds for PIC onset and offset are virtually insensitive to changes in the mAHP-mediating somatic KCa conductance. However, changes in dendritic KCa conductance significantly modulated these threshold values. As dendritic KCa conductance increased, the

Fig. 6 Two-parameter bifurcation elucidating the differential role of somatic and dendritic KCa currents in the control of dendritic PIC. **a, b** A two-parameter bifurcation tracks the steady-state PIC onset and offset for all values of somatic g_{KCaN} (**a**) and dendritic g_{KCaL} (**b**) across a range of I_{SOMA} values. The dashed lines denote the values chosen in the model to reproduce the various firing patterns described in Figs. 2, 3, 4, 5; the black circles denote the corresponding PIC onset and offset on the respective curves

distance marked by the I_{SOMA} range between PIC onset and offset for a given choice of g_{KCaL} value declined and finally became zero suggesting lack of plateau development. *In summary, the dendritic KCa currents strongly modulate the magnitude as well as the onset and offset thresholds of the plateau/PIC while the somatic KCa currents have negligible effect.*

Discussion

KCa mediated PIC control—implications for hyperreflexia

Using a two-compartment SCI motoneuron model, we demonstrate a critical role for dendritic KCa currents in PIC control. In comparison with somatic KCa, the magnitude and thresholds for PIC activation and deactivation were highly sensitive to dendritic KCa (Figs. 4, 5, 6). Removal of dendritic KCa current indeed caused abrupt plateau activation, rapid transition into secondary range and lack of primary range frequencies. However, dendritic KCa showed much less influence on the motoneuron gain than somatic KCa aside from the I–f relation changes just mentioned. In contrast, the mAHP-producing somatic KCa was important for limiting the overall firing frequencies consistent with the inverse relationship observed between

mAHP size and I–f gain found by (Manuel et al. 2006). A previous morphologically detailed computational model of cat motoneurons suggested that the mAHP is important for grading PIC activation (Elbasiouny et al. 2006). Supporting this notion, we did note that a lack of mAHP advanced the secondary range firing corresponding to plateau onset; however, considering the morphological simplification of our model, this grading effect of PICs by the mAHP-producing somatic KCa might be an underestimation. Nonetheless, no change has been noted in the mAHP following SCI (Li and Bennett 2007). Considering these results, we postulate that dendritic L-type calcium-dependent potassium currents are critical for PIC regulation, and a relative reduction of these currents may contribute to the abrupt transitions into secondary and tertiary range frequencies resulting in muscle spasms.

Assumptions of the motoneuron model

Based on available evidence, we made certain assumptions regarding the location of PIC and KCa channels to construct the motoneuron model. The L-type calcium PIC, I_{CaL} is likely mediated by low-threshold Cav1.3 calcium channels that are located in the dendrites (Simon et al. 2003; Ballou et al. 2006; Carlin et al. 2000). Previous modeling reports further support a dendritic origin of the plateau-mediating I_{CaL} that reproduced several experimentally observed motoneuron firing properties such as membrane bistability and I–f hysteresis (e.g., (Booth and Rinzel 1995; Elbasiouny et al. 2006; Grande et al. 2007); however, see (Moritz et al. 2007)). Due to the speed of mAHP generation following an action potential, the mAHP-causing N-type calcium and N-type calcium activated potassium currents were confined to the soma (also see (Booth et al. 1997)). The dendritic location of the L-type calcium-dependent potassium current and its spike-independent activation is based on its insensitivity to calcium N-channel blockers, its sensitivity to calcium L-channel blockers, and the likely dendritic location of the L channels (see “Discussion” in (Li and Bennett 2007)). Moreover, indirect evidence on hippocampal pyramidal cells suggests a sub-type of SK currents potentially of dendritic origin that do not contribute to somatic action potential dependent mAHP (Gu et al. 2008). Secondly, we assumed explicit coupling between the calcium currents (both L- and N-type) and the specific potassium currents that they activate consistent with their spatial separation. But we should note a report on hippocampal neurons which suggests that there is a functional coupling between specific types of calcium (N- and L-type) and calcium-activated potassium (BK and SK, respectively) channels (Marrion and Tavalin 1998). It is conceivable that the selective sensitivity of I_{KCaL} and I_{KCaN} may depend on couplings of L-type and N-type calcium channels with

specific subtypes of SK channels (Liegeois et al. 2003) in addition to, or instead of, distinct localizations.

Motoneurons contain a variety of voltage-activated currents, and several were not included in the model in order to maintain its simplicity and facilitate interpretation of the results. Of particular interest is the hyperpolarization-activated mixed inward cation current (I_h) (Kjaerulff and Kiehn 2001). Increase in I_h following SCI has been reported to elevate resting potential (c.f. (Harvey et al. 2006a)). This may reduce the action potential threshold and accelerate motoneuron recruitment (c.f. (Kiehn et al. 2000)). Furthermore, a potential interaction between I_h and persistent sodium current that activates near spike threshold may promote I_{NaP} -mediated slow repetitive firing (c.f. (Li and Bennett 2003)). However, it is unlikely that such activity could induce spasm-like high frequency discharge in motoneurons (e.g., (Li et al. 2004b)). Available evidence also suggests that elimination of I_h does not alter estimations of 5-HT induced Ca^{2+} PICs after SCI (Li and Bennett 2007). Based on these considerations, we did not include I_h in the current model. Our model also does not take into consideration any potential changes to motoneuron morphology as a significant factor in the induction of hyperexcitability (see (Kurian et al. 2011)). Reports on SCI-induced changes in adult motoneuron morphology are currently inconsistent and changes observed in morphology may be injury and motoneuron specific (Gazula et al. 2004; Bose et al. 2005; Kitzman 2005). Moreover, our two-compartment model provided the simplest means of exploring differences in motoneuron function related to somatic-dendritic channel location; changes in the relative conductance of the two compartments corresponding to SCI-associated changes in morphology should have relatively small impact on the results and conclusions drawn here without redistribution of the voltage- and calcium-dependent conductances.

Mechanism underlying PIC activation and deactivation

The steady state-analysis of Fig. 5 can help explain how the motoneuron discharge is influenced by the dendritic plateau and the two KCa conductances. While the soma is the spike generating compartment, the dendrite contributes to motoneuron firing by providing the additional plateau-mediated depolarization (also see (Booth and Rinzel 1995)). When the current injected into the soma increases during a ramp (Figs. 2, 3), the motoneuron soma reaches firing threshold and the primary range of firing is initiated. Increasing I_{SOMA} also depolarizes the dendrite due to the electrotonic coupling between the two compartments and recruits a dendritic plateau potential. As illustrated in Fig. 5, at I_{SOMA} values corresponding to PIC onset (lower black circle in Fig. 5), the dendritic PICs begin to activate. However, note that the associated plateau depolarization is opposed by the

mAHP causing I_{KCaN} in the soma. This causes a grading effect on PICs and results in an extended primary range firing. We confirm this by comparing Figs. 4a and b, wherein I_{KCaN} removal reduced the extent of primary range and advanced secondary range firing. However, the mAHP itself does not affect either the dendritic plateau magnitude or onset threshold (Fig. 4d, 6a respectively). This is largely due to the distal location of I_{KCaN} from the plateau-mediating PICs. Further increases in I_{SOMA} continue to depolarize dendritic membrane and secondary range of frequencies develops until the plateau is fully activated. The dendritic voltage then stays close to the upper branch of the S-shaped steady-state curve (Fig. 5) and the motoneuron now fires in the tertiary range. During the down ramp, the plateau continues to depolarize the soma. However, the spike generating probability in the soma relies on the balance between the reducing/hyperpolarizing I_{SOMA} and the dendritic depolarization due to the already developed plateau potential. In the absence of dendritic L-type KCa currents, the magnitude of plateau increases and hence causes increases in both the firing frequency and plateau offset threshold (Figs. 4d, c, 6b respectively). As I_{SOMA} further reduces, the depolarization provided by the plateau is insufficient to produce somatic action potentials and firing ends. However, as noted from the S-shaped steady state curve in Fig. 5, the stable range of dendritic voltage on the upper branch remains and the plateau persists. Further hyperpolarization by I_{SOMA} is required for the PICs to be deactivated and the plateau to turn off as indicated by the black circle on the upper branch in the figure. Thus the plateau can outlast somatic firing offset. The stability of the plateau further suggests that a motoneuron could remain depolarized and hence predisposed to begin firing at higher (tertiary) frequencies in response to a somatic/dendritic excitation that would otherwise cause no firing or low frequency firing (also see (Lee and Heckman 1998a, b; Venugopal et al. 2011) for bistability). Hence, the firing offset and the plateau offset are distinct phenomena. Note that the mAHP does not affect plateau offset (Fig. 6a) due to its relative speed and distal location in comparison with the slowly decaying dendritic PICs. However, when the L-type KCa is blocked, the plateau persists for a greater range of hyperpolarizing I_{SOMA} . These model analysis results are consistent with the experimental observations of a lack of PIC deactivation even at a very large hyperpolarizing I_{SOMA} during apamin application (Li and Bennett 2007).

Mechanisms underlying PIC dysregulation after SCI

A variety of alterations occur following spinal cord injury that potentially affect the expression and regulation of PICs and motoneuron excitability. A primary factor is the emergence of constitutive activity of 5HT 2B and 2C

receptors producing recovery of PIC expression and independence of normal neuromodulatory control of PICs (Murray et al. 2010, 2011). Given this state of chronic PIC facilitation, mechanisms that activate or suppress activation of PICs are potentially important factors in causing or reducing motoneuron hyperexcitability. PIC activation may be facilitated by increased excitatory reflexes following spinal cord injury (Baker and Chandler 1987; Li et al. 2004b). Synaptic inhibition has been shown to be a key mechanism in the control of motoneuron discharge and dendritic PIC activation (Bennett et al. 1998; Hultborn et al. 2003), particularly for dendritic sources of inhibition (Bui et al. 2008; Venugopal et al. 2011). Synaptic inhibition may be altered by changes in inhibitory receptors (Bareyre and Schwab 2003; Khristy et al. 2009) and alterations in the strength of segmental inhibitory synaptic inputs (Shapiro 1997; Norton et al. 2008). Moreover, downregulation of the potassium-chloride cotransporter KCC2 following SCI depolarizes the chloride equilibrium potential and reduces the strength of synaptic inhibition (Boulenguez et al. 2010). Thus changes in several mechanisms related to synaptic inhibition may contribute to motoneuron hyperexcitability and hyperreflexia and provide potential targets for therapeutic intervention (Venugopal et al. 2011).

The present work demonstrates the importance of dendritic SK currents in the control of PICs, suggesting that they may be an intrinsic motoneuron target to consider for moderation of motoneuron excitability in addition to 5HT receptors (Murray et al. 2010). If SK channels in somatic and dendritic locations differ only by the source of calcium that activates them, their manipulation would affect I-f gains as well as PIC activation and deactivation. If they differ as well by subtype (Liegeois et al. 2003), their selective manipulation could have therapeutic benefits. The comparative benefits and liabilities of altering the activity of SK channels remain to be explored, but the simulations presented here indicate that this avenue of research merits consideration as a potential therapeutic measure to moderate motoneuron excitability.

Acknowledgments This work was supported by a US National Institutes of Health grant (R01-NS054282) to R.J. and funds from the Barrow Neurological Foundation to T.M.H.

References

- Baker LL, Chandler SH (1987) Characterization of postsynaptic potentials evoked by sural nerve stimulation in hindlimb motoneurons from acute and chronic spinal cats. *Brain Res* 420:340–350
- Ballou EW, Smith WB, Anelli R, Heckman CJ (2006) Measuring dendritic distribution of membrane proteins. *J Neurosci Methods* 156:257–266
- Bareyre FM, Schwab ME (2003) Inflammation, degeneration and regeneration in the injured spinal cord: insights from DNA microarrays. *Trends Neurosci* 26:555–563
- Bennett DJ, Hultborn H, Fedirchuk B, Gorassini M (1998) Synaptic activation of plateaus in hindlimb motoneurons of decerebrate cats. *J Neurophysiol* 80:2023–2037
- Bennett DJ, Li Y, Siu M (2001) Plateau potentials in sacrocaudal motoneurons of chronic spinal rats, recorded in vitro. *J Neurophysiol* 86:1955–1971
- Booth V, Rinzel J (1995) A minimal, compartmental model for a dendritic origin of bistability of motoneuron firing patterns. *J Comput Neurosci* 2:299–312
- Booth V, Rinzel J, Kiehn O (1997) Compartmental model of vertebrate motoneurons for Ca^{2+} -dependent spiking and plateau potentials under pharmacological treatment. *J Neurophysiol* 78:3371–3385
- Bose P, Parmer R, Reier PJ, Thompson FJ (2005) Morphological changes of the soleus motoneuron pool in chronic midthoracic contused rats. *Exp Neurol* 191:13–23
- Boulenguez P, Liabeuf S, Bos R, Bras H, Jean-Xavier C, Brocard C, Stil A, Darbon P, Cattaert D, Delpire E, Marsala M, Vinay L (2010) Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. *Nat Med* 16:302–307
- Bui TV, Grande G, Rose PK (2008) Multiple modes of amplification of synaptic inhibition to motoneurons by persistent inward currents. *J Neurophysiol* 99:571–582
- Carlin KP, Jones KE, Jiang Z, Jordan LM, Brownstone RM (2000) Dendritic L-type calcium currents in mouse spinal motoneurons: implications for bistability. *J Neurosci* 12:1635–1646
- Doedel EJ (1981) AUTO: a program for the automatic bifurcation analysis of autonomous systems. *Congressus Numerantium* 30: 265–284
- Elbasiouny SM, Bennett DJ, Mushahwar VK (2006) Simulation of Ca^{2+} persistent inward currents in spinal motoneurons: mode of activation and integration of synaptic inputs. *J Physiol* 570:355–374
- ElBasiouny SM, Schuster JE, Heckman CJ (2010) Persistent inward currents in spinal motoneurons: important for normal function but potentially harmful after spinal cord injury and in amyotrophic lateral sclerosis. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol* 121:1669–1679
- Ermentrout GB (2002) Simulating, analyzing and animating dynamical systems. SIAM Press, Philadelphia
- Gazula VR, Roberts M, Luzzio C, Jawad AF, Kalb RG (2004) Effects of limb exercise after spinal cord injury on motor neuron dendrite structure. *J Comput Neurol* 476:130–145
- Gorassini MA, Knash ME, Harvey PJ, Bennett DJ, Yang JF (2004) Role of motoneurons in the generation of muscle spasms after spinal cord injury. *Brain* 127:2247–2258
- Grande G, Bui TV, Rose PK (2007) Estimates of the location of L-type Ca^{2+} channels in motoneurons of different sizes: a computational study. *J Neurophysiol* 97:4023–4035
- Gu N, Hu H, Vervaeke K, Storm JF (2008) SK (KCa2) channels do not control somatic excitability in CA1 pyramidal neurons but can be activated by dendritic excitatory synapses and regulate their impact. *J Neurophysiol* 100:2589–2604
- Harvey PJ, Li X, Li Y, Bennett DJ (2006a) 5-HT₂ receptor activation facilitates a persistent sodium current and repetitive firing in spinal motoneurons of rats with and without chronic spinal cord injury. *J Neurophysiol* 96:1158–1170
- Harvey PJ, Li Y, Li X, Bennett DJ (2006b) Persistent sodium currents and repetitive firing in motoneurons of the sacrocaudal spinal cord of adult rats. *J Neurophysiol* 96:1141–1157
- Heckman CJ, Gorassini MA, Bennett DJ (2005) Persistent inward currents in motoneuron dendrites: implications for motor output. *Muscle Nerve* 31:135–156

- Hounsgaard J, Hultborn H, Jespersen B, Kiehn O (1988) Bistability of alpha-motoneurons in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *J Physiol* 405:345–367
- Hultborn H, Denton EM, Wienecke J, Nielsen JB (2003) Variable amplification of synaptic input to cat spinal motoneurons by dendritic persistent inward current. *J Physiol* 552:945–952
- Khristy W, Ali NJ, Bravo AB, de Leon R, Roy RR, Zhong H, London NJ, Edgerton VR, Tillakaratne NJ (2009) Changes in GABA(A) receptor subunit gamma 2 in extensor and flexor motoneurons and astrocytes after spinal cord transection and motor training. *Brain Res* 1273:9–17
- Kiehn O, Kjaerulff O, Tresch MC, Harris-Warrick RM (2000) Contributions of intrinsic motor neuron properties to the production of rhythmic motor output in the mammalian spinal cord. *Brain Res Bull* 53:649–659
- Kitzman P (2005) Alteration in axial motoneuronal morphology in the spinal cord injured spastic rat. *Exp Neurol* 192:100–108
- Kjaerulff O, Kiehn O (2001) 5-HT modulation of multiple inward rectifiers in motoneurons in intact preparations of the neonatal rat spinal cord. *J Neurophysiol* 85:580–593
- Kurian M, Crook SM, Jung R (2011) Motoneuron model of self-sustained firing after spinal cord injury. *J Comput Neurosci*
- Lance JW (1980) The control of muscle tone, reflexes, and movement: Robert Wartenberg Lecture. *Neurology* 30:1303–1313
- Lee RH, Heckman CJ (1998a) Bistability in spinal motoneurons in vivo: systematic variations in persistent inward currents. *J Neurophysiol* 80:583–593
- Lee RH, Heckman CJ (1998b) Bistability in spinal motoneurons in vivo: systematic variations in rhythmic firing patterns. *J Neurophysiol* 80:572–582
- Li Y, Bennett DJ (2003) Persistent sodium and calcium currents cause plateau potentials in motoneurons of chronic spinal rats. *J Neurophysiol* 90:857–869
- Li X, Bennett DJ (2007) Apamin-sensitive calcium-activated potassium currents (SK) are activated by persistent calcium currents in rat motoneurons. *J Neurophysiol* 97:3314–3330
- Li Y, Gorassini MA, Bennett DJ (2004a) Role of persistent sodium and calcium currents in motoneuron firing and spasticity in chronic spinal rats. *J Neurophysiol* 91:767–783
- Li Y, Harvey PJ, Li X, Bennett DJ (2004b) Spastic long-lasting reflexes of the chronic spinal rat studied in vitro. *J Neurophysiol* 91:2236–2246
- Liegeois JF, Mercier F, Graulich A, Graulich-Lorge F, Scuvee-Moreau J, Seutin V (2003) Modulation of small conductance calcium-activated potassium (SK) channels: a new challenge in medicinal chemistry. *Curr Med Chem* 10:625–647
- Manuel M, Meunier C, Donnet M, Zytnicki D (2005) How much afterhyperpolarization conductance is recruited by an action potential? a dynamic-clamp study in cat lumbar motoneurons. *J Neurosci Off J Soc Neurosci* 25:8917–8923
- Manuel M, Meunier C, Donnet M, Zytnicki D (2006) The afterhyperpolarization conductance exerts the same control over the gain and variability of motoneuron firing in anaesthetized cats. *J Physiol* 576:873–886
- Marrion NV, Tavalin SJ (1998) Selective activation of Ca^{2+} -activated K^{+} channels by co-localized Ca^{2+} channels in hippocampal neurons. *Nature* 395:900–905
- Moritz AT, Newkirk G, Powers RK, Binder MD (2007) Facilitation of somatic calcium channels can evoke prolonged tail currents in rat hypoglossal motoneurons. *J Neurophysiol* 98:1042–1047
- Murray KC, Stephens MJ, Ballou EW, Heckman CJ, Bennett DJ (2010) Motoneuron excitability and muscle spasms are regulated by 5-HT2B and 5-HT2C receptor activity. *J Neurophysiol*
- Murray KC, Stephens MJ, Ballou EW, Heckman CJ, Bennett DJ (2011) Motoneuron excitability and muscle spasms are regulated by 5-HT2B and 5-HT2C receptor activity. *J Neurophysiol* 105:731–748
- Norton JA, Bennett DJ, Knash ME, Murray KC, Gorassini M (2008) Changes in sensory-evoked synaptic activation of motoneurons after spinal cord injury in man. *Brain* 131:1478–1491
- Shapiro S (1997) Neurotransmission by neurons that use serotonin, noradrenaline, glutamate, glycine and gamma-aminobutyric acid in the normal and injured spinal cord. *Neurosurgery* 40:168–177
- Simon M, Perrier JF, Hounsgaard J (2003) Subcellular distribution of L-type Ca^{2+} channels responsible for plateau potentials in motoneurons from the lumbar spinal cord of the turtle. *Eur J Neurosci* 18:258–266
- Venugopal S, Crook SM, Hamm TM, Jung R (2009a) A computational study of the interaction between persistent inward currents and recurrent inhibition of alpha motoneurons before and after spinal cord injury. In: 39th Annual Society for Neuroscience Meeting Chicago, IL
- Venugopal S, Crook SM, Kurian M, Jung R (2009b) Role of inhibition in the suppression of alpha motoneuron hyperexcitability following chronic incomplete spinal cord injury. *BMC Neurosci* 10(suppl 1):P343
- Venugopal S, Hamm TM, Jung R (2010) Role of low and high-voltage activated Ca^{2+} dependent K^{+} currents in the control of alpha-motoneuron discharge and its implication in hyperreflexia. *BMC Neurosci* 11:P158
- Venugopal S, Hamm TM, Crook SM, Jung R (2011) Modulation of inhibitory strength and kinetics facilitates regulation of persistent inward currents and motoneuron excitability following spinal cord injury. *J Neurophysiol* 106:2167–2179