Multiple Modes of Amplification of Synaptic Inhibition to Motoneurons by Persistent Inward Currents

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Abstract

The ability of inhibitory synaptic inputs to dampen the excitability of motoneurons is augmented when persistent inward currents (PICs) are activated. This amplification could be due to an increase in the driving potential of inhibitory synapses or the deactivation of the channels underlying PICs. Our goal was to determine which mechanism leads to the amplification of inhibitory inputs by PICs. To reach this goal, we measured inhibitory postsynaptic currents (IPSCs) in decerebrate cats during somatic voltage-clamp steps. These IPSCs were generated by tonic activation of Renshaw cells. The IPSCs exhibited a rapid rise and a slower decay to a plateau level. Activation of PICs always led to an increase in the peak of the IPSC, but the amount of decay after the peak of the IPSC was inversely related to the size of the IPSC. These results were replicated in simulations based on compartmental models of motoneurons incorporating distributions of Renshaw cell synapses based on anatomical observations, and L-type calcium channels distributed as 100- μm -long hot spots centered 100 to 400 μm away from the soma. For smaller IPSCs, amplification by PICs was due to an increase in the driving force of the inhibitory synaptic current. For larger IPSCs, amplification was caused by deactivation of the channels underlying PICs leading to a lesser decay of the IPSCs. As a result of this change in the time course of the IPSC, deactivation of the channels underlying PICs leads to a greater amplification of the total inhibitory synaptic current.

INTRODUCTION

Voltage-gated channels activated by membrane depolarization and mediating inward currents are natural candidates as mechanisms by which a neuron can augment its intrinsic excitability. Their activation by excitatory inputs adds a supplemental source of depolarizing current that either brings the neuron closer to firing threshold or increases its firing frequency. Conversely, there is evidence that the presence of inward currents leads to a greater ability of inhibitory inputs to dampen the excitability of neurons. In neocortical pyramidal neurons, inhibitory postsynaptic potentials (IPSPs) are amplified by the presence of tonically active sodium channels (Hardie and Pearce 2006; Stuart 1999; Williams and Stuart 2003). Dendritic intracellular Ca^{2+} entry, perhaps through P/Q-type channels (Khavandgar et al. 2005; Usowicz et al. 1992), is greatly decreased in Purkinje cells by the

activation of stellate cell inhibitory synaptic inputs situated in the dendrites (Callaway et al. 1995). In spinal motoneurons, the presence of persistent inward currents has been shown to increase the effective strength of inhibitory inputs from two different classes of spinal interneurons: Renshaw cells (Hultborn et al. 2003) and Ia inhibitory interneurons (Kuo et al. 2003).

There are two mechanisms by which inward currents can mediate the amplification of inhibitory synaptic inputs. Hyperpolarization of the membrane potential can lead to the deactivation of the inward current. The resulting loss of the depolarizing current is equivalent to adding a hyperpolarizing current. Alternatively, the activation of inward currents depolarizes the membrane, thereby increasing the driving potential of the inhibitory synapses. By increasing the driving potential of inhibitory synapses, inward currents increase the magnitude of the hyperpolarizing current through the postsynaptic ligand-gated channels. These two mechanisms are distinct. One requires the deactivation of the inward currents, whereas the other is a biophysical consequence of the presence of the inward currents. The continual entry of cations such as Ca^{2+} through voltage-gated channels can lead to the activation of various biochemical pathways (Bootman et al. 2001; Miller 1987), and therefore the particular mechanisms by which inhibitory inputs are strengthened by inward currents may have different long-term effects on the physiological properties of a neuron.

Persistent inward currents (PICs) in motoneurons are composed of a Ca^{2+} current mediated by L-type Ca^{2+} channels ($\text{Ca}_v1.3$ subtype) and a persistent sodium current of unknown origin (Carlin et al. 2000; Heckman et al. 2003; Li and Bennett 2003; Li et al. 2004). As previously mentioned, PICs have been linked to the amplification of inhibitory inputs to motoneurons. Our goal was to determine which biophysical mechanism is responsible for the amplification of inhibitory inputs to motoneurons. Electrophysiological experiments were conducted using the feline decerebrate preparation. PICs were activated in hindlimb motoneurons using a somatic voltage clamp, whereas synaptic inhibition was elicited through tonic activation of Renshaw cells. In some cells, the amplification was accompanied by a change in the time course of the inhibitory postsynaptic currents (IPSCs), whereas in other cells the time course of the IPSCs was fixed. The change in the time course of the IPSCs led to a greater amplification of the total inhibitory synaptic current. These features were also observed in compartmental models of motoneurons incorporating realistic morphological characteristics of motoneurons, distribution of synaptic inhibition to motoneurons, and distribution of L-type calcium channels. Simulations using the compartmental models showed that amplification in the absence of changes in the time course of the IPSCs was due to an increase in the driving potential, whereas amplification involving a change in the time course of the IPSCs resulted from the persistent deactivation of the channels by synaptic inhibition. The persistent deactivation of the channels was produced by increasing inhibitory synaptic conductance. These results suggest that in the presence of PICs, the strength of an inhibitory synaptic input will determine the activation state of the channels underlying PICs and this may alter the time course of the synaptic inhibition. Portions of this work were previously presented in abstract form (Bui et al. 2006a).

METHODS

Animal preparation

The experimental protocols were conducted in compliance with approved institutional protocols (Queen's University Animal Care Committee) and in accordance with Canadian Council of Animal Care. All experiments were conducted on adult cats.

Following induction of anesthesia with ketamine (6.25 mg/kg) and midazolam (0.31 mg/kg), a laminectomy was performed to expose the L₄ to S₂ segments of the spinal cord under deep gaseous anesthesia (2–3% isoflurane). Decerebration was performed by ligating both common carotid arteries, transecting the midbrain at the junction of the inferior and superior colliculi, and aspirating the entire forebrain. The calvarium was packed with gauze soaked with saline and thrombin to reduce blood loss. On completion of the decerebration, isoflurane was discontinued.

In the left hindlimb, the following nerves was isolated and placed on hook-shaped bipolar electrodes: sciatic nerve (Sc), tibial nerve (Ti), lateral gastrocnemius (LG), medial gastrocnemius (MG), and common peroneal (CP). Dorsal roots from L₅ to S₁ were cut on the left side of the spinal cord. The animals were paralyzed with gallamine triethiodide (Flaxedil, 2.5–5 mg · kg⁻¹ · h⁻¹, administered intravenously; Poulenc Laboratories) and ventilated using a respirator to maintain an end-tidal CO₂ of 3.5–4.5%.

Intracellular recordings of motoneurons from L₅ to L₇ were obtained using sharp glass microelectrodes with tip diameters broken to 1.8–2.0 μm and filled with 2.5 M potassium acetate. Within this range of tip diameters, the resistances of the electrodes were typically 3–5 MΩ on the surface of the spinal cord. These resistances usually increased to 4–8 MΩ as the electrode was advanced to the ventral horn. The voltage clamp was applied using an Axoclamp 2A amplifier (Axon Instruments) in single-electrode discontinuous mode with a sampling frequency of 8–10 kHz. An external circuit was added to enhance the low-frequency gain of the feedback loop 11-fold to minimize mismatches between the command voltage and the actual voltage as described in Heckman and Lee (2001). Typically the difference between the two was <10% in our recordings.

Electrophysiological protocol

Voltage-clamp steps were applied in intervals of 5 mV beginning at –5 mV and increasing to 30 mV relative to the resting membrane potential. Each step was 2.5 s long and was repeated three times at intervals of 5 s. The voltage-clamp step was divided into five consecutive 500-ms epochs (E1–E5). No inhibition was activated during E1. Synaptic inhibition was activated during E2 and E3 or during E3 and E4. In one motoneuron, two sources of inhibitory inputs were available, in which case the first source of inhibition was activated during E2 and E3 and the second source of inhibition was activated during E3 and E4. Thus in this cell there was a single source of inhibition during E2; concurrent activation of two different sources of inhibition during E3 and a single source of inhibition during E4. Similar to E1, there was no inhibition during E5.

Sources of synaptic inhibition

Ipsilateral hindlimb nerves were stimulated at two- to fivefold motor threshold to recruit recurrent inhibition from Renshaw cells (Eccles et al. 1954; Renshaw 1946). Stimulation frequency was set to 50 Hz. At these stimulation frequencies, IPSCs consisted of high-frequency transients superposed on a lower-frequency component consisting of an initial rapid rise to peak within 50–200 ms followed by a slower decay to a plateau (Figs. 1A and 2A).

Data analysis

Data were accepted only from cells in which the holding current applied at the onset of the voltage clamp (0 nA at the resting membrane potential) did not increase by >10 nA (typically 2–4 nA) and the resting membrane potential was more hyperpolarized than –55 mV throughout the period of data collection. To determine the magnitude of the total current generated by the leak currents, PICs, and IPSCs without the influence of fast transients associated with each stimulus to the nerve, the experimental recordings were low-pass filtered with a cutoff frequency of at most half the frequency of the nerve stimulation to remove high-frequency components without removing all of the low-frequency components of interest (i.e., the rapid rise and slower decay to plateau). This was implemented by a moving-median window whose width was at least twice the period of the synaptic inhibition (for 50-Hz stimulation of Renshaw cells, the width would thus be at least $2 \times 20 \text{ ms} = 40 \text{ ms}$) using the running-median procedure in Sigmaplot (Version 8.0, SPSS). Subsequently, a linear interpolation was performed using the last 200 ms of E1 and the last 200 ms of E5 to estimate the current in the absence of synaptic activation. This current was subtracted from the recorded current to estimate the effective current produced by the IPSCs during the voltage clamp.

Due to the limited spatial extent of the voltage clamp, the driving force at synapses located further away from the cell body may not be adequately clamped. Therefore the activation of an inward current, such as PICs, located in the dendrites may depolarize certain regions of the dendritic trees to levels above the magnitude of the voltage clamp, thereby resulting in a supralinear scaling of the amplitude of the IPSCs. To quantify amplification of the IPSCs by PICs, we performed a linear regression on the peak of the IPSCs generated at holding membrane potentials from –5 to 10 mV, which was sub-threshold to the activation of PICs in all motoneurons reported. A linear extrapolation based on this regression was then performed to estimate the peak of the IPSCs in the absence of PICs, and the ratio between the peak of the largest IPSCs recorded and the estimated peak was used as the ratio of amplification of the peak of the IPSCs. A similar methodology was used to estimate the ratio of amplification of the time integral of the IPSCs.

Motoneuron model

To examine the biophysical mechanisms underlying the amplification of synaptic inhibition during concurrently activated PICs, we used compartmental models based on morphological measurements of intracellularly stained feline neck motoneurons. The value of the specific resistivity of the cytoplasm (R_i) selected for this study, $70 \Omega \cdot \text{cm}$, is based on calculations for motoneurons (Barrett and Crill 1974) and is close to the value for saline (Hille 2001). In

previous motoneuron modeling studies (Bui et al. 2003, 2006b), the value of the specific resistivity of the membrane (R_m) was set to $15,000 \Omega \cdot \text{cm}^2$. In the simulations presented herein, we set R_m to be $5,000 \Omega \cdot \text{cm}^2$ to mimic the presence of background synaptic activity or leak potassium conductances that may have been present during our electrophysiological recordings. Simulations performed with an R_m value of $15,000 \Omega \cdot \text{cm}^2$ replicated the main findings but with larger IPSCs and PICs.

L-type calcium channels were distributed in discrete regions in the dendrites (Bui et al. 2006b; ElBasiouny et al. 2005) that were $100 \mu\text{m}$ long and centered 100 to $400 \mu\text{m}$ away from the cell body. This distribution is based on theoretical analysis of experimental observations made by Bennett et al. (1998), where the threshold of activation of plateau potentials in motoneurons as seen at the soma is depolarized by the presence of synaptic inhibition and hyperpolarized by synaptic excitation. An analysis of the membrane potential distribution just subthreshold to the activation of the plateau potentials in the presence and absence of synaptic activity led to the conclusion that the channels mediating the plateau potentials were found in the discrete regions in the dendritic tree. Modeling studies of this distribution supported its validity. Further analysis and elaboration on this distribution can be found in Bui et al. (2006b). The conductance of L-type Ca^{2+} channels $g_{\text{L,Ca}}$ was modeled as

$$g_{\text{L,Ca}} = g_{\text{peak,Ca}} m \quad (1)$$

Here $g_{\text{L,Ca}}$ represents the maximal conductance per surface area. The variable m is a voltage- and time-dependent activation variable, described by the differential equation

$$\frac{dm}{dt} = \frac{m_{\infty} - m}{\tau_m} \quad (2)$$

where the time constant of activation τ_m was assigned a value of 20 ms (Carlin et al. 2000). The steady-state activation level m_{∞} is given by

$$m_{\infty} = \frac{1}{1 + e^{-(V_m - V_{1/2})/k}} \quad (3)$$

where the half-activation voltage $V_{1/2}$ was -33 mV (in proximity to values used by Booth et al. 1997; Carlin et al. 2000; Svirakis et al. 2001) and the activation sensitivity k was assigned a value of -6 mV (Carlin et al. 2000). E_{rev} for the calcium current was set at 60 mV (Carlin et al. 2000).

Glycine/GABAergic synapses were modeled as conductance changes with a single time constant rising phase and a dual-component decay with two time constants based on observed dual glycinergic and GABAergic unitary IPSCs on motoneurons (Jonas et al. 1998) as well as dual glycinergic and GABAergic IPSPs on Renshaw cells (Schneider and Fyffe

1992). The rationale for the kinetics of these synapses is further explained in Bui et al. (2005). Unitary transient conductance changes were modeled as a piecewise function

$$g_{\text{gly/GABA}}(t) = \begin{cases} \frac{g_{\text{peak}} t e^{-t/t_{\text{peak}}}}{t_{\text{peak}}} & t < t_{\text{peak}} \\ g_{\text{peak}} [A e^{-(t-t_{\text{peak}})/\tau_1} + B e^{-(t-t_{\text{peak}})/\tau_2}] & t > t_{\text{peak}} \end{cases} \quad (4)$$

where the two constants A and B, associated with each decay component, are 0.74 and 0.26, respectively. Since the shape of unitary IPSCs resembled the shape of composite IPSCs produced by sustained simulation of motor axons at frequencies >50 Hz (cf. Lindsay and Binder 1991; Maltenfort et al. 2004; Figs. 1A and 2A), we modeled the conductance caused by this type of input as a time-dependent, composite conductance rather than as a train of unitary conductance changes. Thus the time course of conductance change of each synapse was described by Eq. 1 and the time-to peak t_{peak} and the two decay time constants, τ_1 and τ_2 , were set at 100, 50, and 1,400 ms, respectively, to replicate the experimentally observed composite IPSCs (compare Figs. 1 and 2 to Fig. 6). The peak conductance g_{peak} was adjusted from 3 to 6 pS. At 6 pS, IPSCs at rest had a peak of 3.0 nA, which was close to the peak IPSC recorded experimentally at rest (3.1 nA). The glycine/GABA synapse mediates a chloride-mediated current with a reversal potential value ($V_{\text{gly/GABA}}$) of -81 mV, as calculated by Stuart and Redman (1990) for glycinergic IPSCs in in vivo motoneurons.

The glycine/GABA synapses were distributed based on the observed distribution of Renshaw cell synapses on motoneurons (Fyffe 1991). More specifically, synapses were distributed uniformly at a density of one synapse/1,000 μm^2 between 65 and 470 μm away from the cell body.

RESULTS

Stable intracellular recordings were obtained from seven animals. The current generated by recurrent inhibition was measured during voltage-clamp steps to different holding potentials (Figs. 1 and 2). IPSCs consisted of high-frequency transients superimposed on a lower-frequency component that was extracted by a low-pass filter. The lower-frequency component of IPSCs produced by recurrent inhibition usually rose within the first 50–200 ms and then decayed to a plateau (Figs. 1A and 2A).

In the absence of activation of intrinsic currents, the current injected during the first 500 ms of the voltage-clamp step should scale linearly with the magnitude of the step. The activation of a net inward current is revealed by a sublinear increase in the current injected by the voltage clamp as the holding potential from rest is increasingly depolarized, resulting in a negative slope in the relation between current injected by the voltage clamp and the holding potential (Figs. 1, B and C and 2, B and C). The currents associated with the fifth voltage step shown in Figs. 1A and 2A illustrate two examples of sublinear increases in the current injected by the voltage clamp. We attributed these sublinear increases to PICs (Hounsgaard et al. 1984; Lee et al. 2003; Li and Bennett 2003; Schwandt and Crill 1980).

In 11 motoneurons, 12 sets of IPSCs (in one motoneuron, IPSCs were generated by two putatively different populations of Renshaw cells activated for 1,000 ms with a delay of 500 ms between the activation of the first population and the activation of the second population; see METHODS) were measured with concurrent activation of PICs (Figs. 1*D* and 2*D*). In each of these motoneurons, the activation of PICs amplified the magnitude of IPSCs as measured by the peak of the IPSCs (Figs. 1*E* and 2*E*). The average percentage increase of the peak of the IPSC by activation of PICs was $83.4 \pm 45.5\%$.

Change in the time course of IPSCs by activation of PICs

As mentioned earlier, IPSCs generated by the activation of Renshaw cells through stimulation of motor axons have a time course consisting of a rising phase followed by a decay to a plateau phase. The magnitude of the plateau in relation to the peak of the IPSC was found to change to varying degrees by the activation of PICs. To better illustrate the changes in the time course of the IPSCs by PICs, we normalized the two sets of IPSCs from the motoneurons in Figs. 1 and 2 (Fig. 3). The IPSCs recorded from the motoneuron in Fig. 1 illustrate a case where there was little change in the time course of the IPSC (normalized IPSCs in Fig. 3*A*). This type of amplification was termed “isomorphic.” The IPSCs recorded from the motoneuron in Fig. 2 illustrate a case where there was a large change in the time course of the IPSC (normalized IPSCs in Fig. 3*B*). More specifically, the decay following the rising phase was reduced when the IPSC was amplified by the activation of PICs. This type of amplification was termed “pleomorphic.”

The data shown in Figs. 1 and 2 are two representative cases of isomorphic and pleomorphic amplification. The amount of decay following the peak of the IPSC was measured as the size of the plateau (time-average of IPSC in the last 500 ms of motor axon stimulation) in relation to the peak of the IPSC. In each set of IPSCs we calculated the change of the plateau in the IPSC generated during the largest PICs (which we will term “amplified IPSC”) in relation to the plateaus of IPSCs recorded from -5 to 10 mV (which we will collectively term as “subthreshold IPSC”). The average increase (which we will simply term as “the increase”) in the IPSC plateau of the amplified IPSC and each of the subthreshold IPSCs was calculated (Fig. 4). The increase in the IPSC plateau by PICs ranged from 4.9 to 129% and was found to be correlated to the peak of the amplified IPSC ($r = 0.89$; F -test, $P < 0.05$). In other words, isomorphic amplification was observed for IPSCs with smaller peaks, whereas pleomorphic amplification was observed for IPSCs with larger peaks.

Considering that the presence of PICs has different effects on the time course of IPSCs, we would expect that the amplification of the entire IPSC could differ from the amplification of the peak of the IPSC among the measured sets of IPSCs. We examined the amplification of the peak or of the time integral of the amplified IPSC in relation to the increase in the IPSC plateau (Fig. 5). Indeed, although the amplification of the peak of the IPSC varied little with the change in the IPSC plateau ($r = -0.06$; F -test, $P > 0.5$), the amplification of the integral of the IPSC varied with the change in the IPSC plateau ($r = 0.74$; F -test, $P < 0.05$). The slopes of the two regression lines were found to be significantly different using a t -test with $P < 0.05$.

Biophysical mechanisms underlying the amplification of IPSCs

The correlation between the change in the time course of the IPSCs by PICs and the size of the IPSC suggests that the form of amplification of the IPSC by PICs is dependent on the size of the conductance underlying the IPSC. To investigate the biophysical mechanisms that may underlie the two forms of amplification, we used compartmental models of motoneurons (see METHODS).

To mimic the electrophysiological procedures of our experiments, we applied the same protocol to the compartmental models (see Fig. 6). Voltage-clamp steps of 2.5-s duration from -5 to 30 mV from rest (-64 mV) were applied and recurrent inhibition was activated $1,000$ ms from the onset of the voltage-clamp step. The magnitude of the conductance change of each synapse was varied to assess whether increasing the size of IPSCs could lead to a shift from isomorphic amplification to pleomorphic amplification. First the current reaching the cell body in the absence of IPSCs was measured to determine the activation of PICs (Fig. 6A). As seen by the sublinear increase in current between the 10 - and 15 -mV voltage-clamp steps, PICs were first activated at 10 mV. The IPSC was measured by subtracting the current injected by the voltage clamp in the absence of inhibitory synaptic activity (in the first 500 ms of the voltage clamp) from the current injected by the voltage clamp during inhibitory synaptic activity (Fig. 6B). To assess the form of amplification, we normalized the IPSCs to the peak of the largest IPSC (Fig. 6C). At the smallest g_{peak} modeled, the amplification is isomorphic with the maximal increase in IPSC plateau measured at 20% (measured for the IPSC at the 20 -mV voltage-clamp step). As the value of g_{peak} increases, the amplification becomes pleomorphic with the maximal increase in IPSC plateau measured at 86% (measured for the IPSC at the 20 -mV voltage-clamp step), as illustrated qualitatively by the larger plateau of the amplified IPSCs in relation to the plateau of the other IPSCs when they are all normalized to the peak.

To determine whether there are differences in the level of channel deactivation underlying the different forms of amplification, we measured the probability of channel opening of each L-type Ca^{2+} channel hot spot (Fig. 7, A and B) during the largest IPSCs (generated during the 20 mV from rest voltage-clamp step) in the model with $g_{\text{peak}} = 3$ and 6 nS (Fig. 7C). Before the activation of inhibitory synapses, 25 of 34 hot spots were active as defined by a probability of channel opening >0.5 . However, in the model with $g_{\text{peak}} = 6$ nS, there was a greater number of hot spots whose probabilities of channel opening were brought to levels <0.5 by the synaptic inhibition. To better illustrate the differences in the probability of channel opening in the two models, we calculated the percentage of hot spots that were activated during the period of synaptic inhibition (Fig. 7D). As seen in Fig. 7D, there were more hot spots deactivated when the inhibitory conductance was greater. The IPSC in the model with $g_{\text{peak}} = 6$ nS exhibits multiple plateaus of decreasing magnitudes. Note that each decrease from one plateau to another corresponds to a reactivation of a number of hot spots. This suggests that if the number of deactivated hot spots at the peak of the IPSC had remained the same as the inhibitory conductance decreased, then the IPSC would not have exhibited a decay and would have reached a plateau at the same level as the peak, in which case the IPSC would have truly undergone pleomorphic amplification. Conversely, if all the hot spots that had deactivated at the peak of the IPSC had reactivated as the inhibitory

conductance decreased, then the IPSC would have undergone isomorphic amplification. Therefore the preceding simulations suggest that the two different forms of amplification of IPSCs by PICs are produced by different levels of channel deactivation. When synaptic inhibition is weaker, amplification of IPSCs is produced by the increase in the inhibitory driving potential resulting from the activation of PICs, which is instantaneous and therefore does not engender a change in the shape of PICs. With stronger synaptic inhibition, amplification of IPSCs is produced by the persistent deactivation of at least some of the channels underlying PICs. Since the processes of channel deactivation and reactivation are not instantaneous, the IPSCs undergo a change in shape.

Considering the different reported kinetics of L-type Ca^{2+} channels (Carlin et al. 2000; Helton et al. 2005; Li and Bennett 2003), we repeated these simulations by increasing the time constant of activation of L-type Ca^{2+} channels in our models to 40 and 80 and 200 ms, respectively. When g_{peak} was set to 6 nS the maximal increase in IPSC plateaus by the activation of PICs was 135, 65, and 32%, respectively, when the time constant was set to 40, 80, and 160 ms (Fig. 7E).

Finally, to assess whether there were properties of the hot spots that made them susceptible to deactivation, we compared the number of inhibitory synapses within 0.1λ of the hot spots, the electrotonic distance from the cell body and the passive input resistance (Fig. 8). All of these measurements were taken using the center of the hot spot as the point of reference. On average, the hot spots that were deactivated had a greater number of inhibitory synapses within 0.1λ (1.8 ± 0.5 vs. 1.3 ± 0.6), were electrotonically closer to the cell body (0.32 ± 0.5 vs. 0.38 ± 0.8), and were in areas with lower local passive input resistance (28.4 ± 21.4 vs. $38.8 \pm 22.8 \text{ M}\Omega$). Of these three measures, only the number of inhibitory synapses within 0.1λ was significantly different (Mann–Whitney test: number of synapses within 0.1λ , $P < 0.05$; electrotonic distance, $P > 0.1$; input resistance, $P > 0.23$), suggesting that the strength of the inhibition was the most critical determinant of whether an active hot spot is deactivated by synaptic inhibitory activity, although we cannot completely discount the contribution of the other two properties.

DISCUSSION

Activation of PICs by excitatory inputs to motoneurons produces an intrinsic source of depolarization that increases the excitability of motoneurons. In light of their depolarizing action, PICs were initially regarded as a means of amplifying excitatory inputs to motoneurons (Hounsgaard et al. 1984, 1988; Lee and Heckman 2000; Lee et al. 2003; Schwindt and Crill 1980). However, PICs have also been shown to increase the ability of inhibitory synaptic activity to decrease the excitability of motoneurons. The current produced by reciprocal inhibition through activation of Ia inhibitory interneurons (Hultborn et al. 1968) increases in the presence of PICs (Kuo et al. 2003) and the decrease in firing frequency following activation of recurrent inhibition through activation of Renshaw cells also increases in the presence of PICs (Hultborn et al. 2003). The exact biophysical mechanisms underlying the reported amplification of inhibitory inputs to motoneurons have never been determined. Amplification of inhibitory inputs could occur either by an increase in the driving potential at inhibitory synapses by PICs or by deactivation of the channels

underlying PICs. We present voltage-clamp recordings and computer simulations of motoneurons to show that these two mechanisms of amplifying synaptic inhibition lead to different effects on the time course of inhibitory synaptic currents evoked by activation of Renshaw cells. Steady-state IPSCs from Renshaw cells initially rise to a peak and then decay to a plateau. Amplification of IPSCs by PICs involving an increase in the inhibitory driving potential increased the size of the IPSC, whereas its time course remained fixed. Amplification of IPSCs involving a deactivation of the channels underlying PICs increased the size of the IPSC and, in addition, the time course of the IPSC changed as the magnitude of the plateau relative to the peak also increased. That is, the persistent deactivation of the channels decreased the amount of decay of the IPSCs, leading to a greater amplification of the IPSCs when considered on the basis of the time integral rather than of the peak magnitude of the hyperpolarizing current generated by the synaptic activity.

Conditions for deactivation of PICs

Our observations suggest that the amplification of inhibitory inputs to motoneurons by PICs can be mediated by an increase in the inhibitory driving potential or through the persistent deactivation of the channels underlying PICs. Both the sodium and calcium components of the PICs are activated by membrane depolarization (Li and Bennett 2003). To persistently deactivate these currents, a sufficient membrane hyperpolarization must be produced to offset the combination of any applied membrane depolarization (intrinsic or synaptic) and the membrane depolarization produced by the PICs themselves. Thus the ability of inhibitory synaptic inputs to deactivate the channels underlying PICs must depend on the strength of the inhibitory inputs in relation to the summed strength of the various sources of depolarizing currents.

PROXIMITY TO SOMATIC QUASI-VOLTAGE CLAMP—In addition to the channels underlying PICs, an important source of intrinsic depolarization comes from the channels underlying the repetitive firing of action potentials. During repetitive firing of action potentials, the membrane potential of the soma is effectively voltage clamped (Koch et al. 1995), which is analogous to the somatic voltage clamp that we applied. There are two characteristics of this quasi-somatic voltage clamp to take into consideration. First, the average membrane potential during repetitive firing increases with the frequency of firing (Koch et al. 1995) and, in motoneurons, this increase has been measured to be in the order of 10 to 15 mV (Schwindt and Crill 1982). Therefore at higher firing rates, the magnitude of the depolarization applied by the somatic voltage clamp would be greater, which would increase the magnitude of the PICs and the required membrane hyperpolarization applied by synaptic inhibition to deactivate the channels underlying PICs to produce pleomorphic amplification. However, due to the large electrotonic size of motoneurons (Bui et al. 2003), a voltage clamp at the soma does not clamp the entire dendritic tree (Heckman and Lee 2001). Our observations of different levels of channel deactivation among our motoneuron samples may be a reflection of varying locations of L-type Ca^{2+} channels away from the cell body and/or a reflection of varying spatial extents of the somatic voltage clamp across the motoneuron population. In our analysis of the properties of hot spots that were either deactivated or not deactivated, there was a trend toward those channels that were within a certain distance of the somatic voltage clamp being more easily deactivated by inhibitory

synapses because the clamp may limit the magnitude of the regenerative current generated by the L-type Ca^{2+} channels. A recent modeling study (Grande et al. 2007) suggests that L-type Ca^{2+} channel hot spots are located further away from the cell body in larger motoneurons. Thus the channels in smaller motoneurons may be more susceptible to deactivation because they are located in areas under greater control from the somatic voltage clamp.

PROXIMITY OF SYNAPTIC INPUTS AND PICS—In addition to the proximity of the channels to the soma and the somatic voltage clamp imposed by repetitive firing, another factor to consider is the proximity of the channels to the inhibitory synaptic inputs. In light of the superposition between the inhibitory synapses originating from Renshaw cells and the L-type Ca^{2+} channels (see *Validity of assumptions used to construct the compartmental models*), Renshaw cells are well positioned to control the level of activation of PICS. Other systems of inhibitory synapses to motoneurons may not be distributed like the system of Renshaw cell inputs. Inhibitory synapses from Ia inhibitory interneurons are believed to be located closer to the soma (Burke et al. 1971; Fyffe 2001). As well, the size of gephyrin clusters, a postsynaptic glycine receptor clustering protein, increases with distance away from the cell body. Systems of inhibitory inputs that are more proximal or more distal to the L-type Ca^{2+} channels may not be optimally located to deactivate PICS and undergo pleomorphic amplification.

KINETICS OF SYNAPTIC CONDUCTANCE CHANGE AND L-TYPE Ca^{2+} CHANNELS—As the time activation of the L-type Ca^{2+} channels was increased to >40 ms in our simulations, the change in the IPSC plateau size decreased. One possible explanation is that there is a window beyond which the kinetics of activation and inactivation of the channels become too slow for the IPSC to deactivate a sufficient amount of channels before the IPSC decays. The lack of channel deactivation would preclude any change in the IPSC plateau size.

Validity of assumptions used to construct the compartmental models

Our models were based on several assumptions: L-type Ca^{2+} channels were distributed as hot spots; the inhibitory synapses were distributed according to the anatomical observations of Fyffe (1991) describing the distribution of Renshaw cell inputs to motoneurons; PICS were mediated solely by calcium channels; and neck motoneurons represent a valid model for hindlimb motoneurons. How critical were these assumptions in terms of the ability of our model to accurately describe the mechanisms responsible for the two forms of amplification of IPSCs by PICS seen experimentally?

DISTRIBUTION OF L-TYPE Ca^{2+} CHANNELS—The exact distribution of the L-type Ca^{2+} channels remains unresolved. Different distributions have been suggested from observations using immunohistological methods (Ballou et al. 2006; Carlin et al. 2000; Simon et al. 2003; Westenbroek et al. 1998) or using different computational methods (Booth et al. 1997; Bui et al. 2006a; Carlin et al. 2000; ElBasiouny et al. 2005; Taylor et al. 2004). The distribution of L-type Ca^{2+} channels as discrete regions (hot spots) in the dendrites of the motoneuron was based on a heuristic construct that compared the membrane

potential throughout the dendritic tree prior to activation of PICs in the absence of synaptic activity and the presence of synaptic excitation or inhibition (Bui et al. 2006b). This construct led to the prediction that L-type Ca^{2+} channels were distributed in hot spots located 100 to 400 μm away from the cell body. Models equipped with 100- μm -long hot spots at these locations are able to replicate the changes in somatic threshold of PICs due to tonic excitatory or inhibitory synaptic activity (Bennett et al. 1998). Therefore this distribution was used in the present study. Another recent modeling study also concluded that L-type Ca^{2+} channels were located in discrete regions in the dendritic tree of motoneurons (ElBasiouny et al. 2005). As a consequence of this more punctate distribution, the L-type Ca^{2+} channels are closer to the inhibitory synapses from Renshaw cells. According to Fyffe (1991), about 93% of the synapses from Renshaw cells are found between 65 and 470 μm away from the cell body of motoneurons. This superposition may have increased the ability of the inhibitory synapses to deactivate the channels, leading to pleomorphic amplification. An additional consequence of a punctate distribution of L-type Ca^{2+} channels in our models is that it allows for amplification of synaptic inhibition not only by increases in driving potential but also by persistent deactivation of the channels to occur within the same motoneuron. At the level of a single compartment innervated by a single population of inhibitory synapses, both mechanisms are mutually exclusive; however, both mechanisms of amplification can be present at different spatially separated segments of the dendritic tree of an individual motoneuron. This is another possible explanation as to why amplification of IPSCs by PICs was not always strictly isomorphic or pleomorphic, and why we observed different levels of change in the time course of the IPSCs following amplification by PICs.

LACK OF PERSISTENT SODIUM CURRENT—Persistent sodium channels were not included in the model due to the lack of information regarding their possible location on the dendritic tree of motoneurons. The effect of including persistent sodium channels in our models would depend on their location in relation to the somatic voltage clamp. If the source of the persistent sodium current is somatic, then the generation of this current will be controlled solely by the somatic voltage clamp. If the source of the persistent sodium current is axonal, then its separation from the dendritic tree will also preclude its participation in the amplification of inhibition from Renshaw cells. We would predict that if the channels underlying the persistent sodium current were found in the dendritic tree, the deactivation of these channels could also lead to pleomorphic amplification in light of the rapid kinetics of activation and deactivation of the persistent sodium component of PICs (Li and Bennett 2003).

SIZE OF PICS—Despite the absence of a sodium component, the magnitude of the PICs in our model reached to >40 nA. In recordings of motoneurons, PICs in the order of 30 nA have been reported (Fig. 3B of Kuo et al. 2003). The larger magnitude may be due in part to the lack of outward currents in our model. It may also be indicative of an overestimation of the number of L-type Ca^{2+} channels, which may facilitate the appearance of pleomorphic amplification through channel deactivation. Conversely, a greater PIC leads to greater membrane depolarization that synaptic inhibition has to overcome to deactivate the L-type Ca^{2+} channels, thus creating harder conditions for pleomorphic amplification to occur. Thus

it is not readily apparent whether greater PICs in our model facilitated or prevented pleomorphic amplification.

VALIDITY OF USING NECK MOTONEURONS TO STUDY HINDLIMB

MOTONEURONS—The models in this study were based on reconstructions of neck motoneurons because detailed geometrical measurements of this group of motoneurons were available from past studies (e.g., Bui et al. 2003). As discussed in Bui et al. (2003), the morphological properties of our sample of motoneurons are similar to those of hindlimb motoneurons in terms of size and branching pattern, and therefore we believe that using these neck motoneurons as surrogate for hindlimb motoneurons is valid in this study.

Alternative biophysical mechanisms

We cannot discount the possibility that intrinsic outward currents could lead to the change in the time course of the IPSC. These outward currents could be activated by increased intracellular Ca^{2+} concentration (e.g., Ca^{2+} -activated K^+ current) by way of the L-type Ca^{2+} channels or by membrane repolarization since it occurred only after the peak of the IPSC. Ca^{2+} -dependent potassium currents are known to be colocalized with L-type Ca^{2+} channels (Lipscombe et al. 2004), and SK channels are present in motoneurons (Grunnet et al. 2004). They could be activated by increased Ca^{2+} concentration by activation of PICs. Alternatively, the net outward current could result from the deactivation of an inward current that is independent of the presence of inhibitory synaptic activity. This would include Ca^{2+} -dependent inactivation of L-type Ca^{2+} channels (Lipscombe et al. 2004) or slow inactivation of the Na^+ component of PICs (Li and Bennett 2003). However, if any of these mechanisms is responsible for the pleomorphic amplification of IPSCs, then their timing must coincide precisely with the peak of the IPSC (that is, 1,050–1,150 ms after the start of the voltage-clamp step) to cause the changes in the time course that were observed.

Physiological implications

On a conceptual level, both forms of IPSC amplification by PICs lead to an increased ability of inhibitory synapses to decrease the excitability of motoneurons. However, pleomorphic amplification leads to a greater amplification in terms of total negative current generated by the IPSC by reducing the decay of the IPSC. The pleomorphic form of amplification may also have longer-lasting effects on the excitability and biochemical state of motoneurons. Deactivation of the channels underlying PICs prevents further calcium entry through L-type Ca^{2+} channels. Calcium entry through L-type Ca^{2+} channels leads to the activation of biochemical pathways mediated by changes in intracellular calcium concentrations (e.g., Thiagarajan et al. 2005; Yasuda et al. 2003). Perrier et al. (2000) proposed that plateau potentials in turtle motoneurons were facilitated by calcium-calmodulin-dependent pathways. Thus the persistent deactivation of L-type Ca^{2+} channels may reduce the probability of further activation of PICs. As well, the calcium-buffering capacity of motoneurons has been shown to be low in comparison to other neurons of the nervous system, suggesting that the dynamics of cytosolic Ca^{2+} concentration changes in motoneurons are rapid (Palecek et al. 1999). Changes in cytosolic Ca^{2+} concentration have been linked to the regulation of synaptic plasticity (Jeong et al. 2006) or motoneuron homeostasis (Dayanithi et al. 2006). Therefore the implications of isomorphic or

pleomorphic amplification of inhibitory synapses may not be limited to differences in the magnitude of the effective inhibitory synaptic current generated but could extend to long-lasting changes in motoneuron excitability.

Acknowledgments

We thank M. Neuber-Hess, R. Cranham, S. Armstrong, S. Gordon, and Dr. Steve Iscoe for invaluable assistance during the surgery and A. Pollett, S. Woods, S. Cushing, M. Ter-Mikaelian, and D. Pace for assistance with the in-house computer software.

GRANTS

This work was supported in part by the Canadian Institutes of Health Research. T. V. Bui was supported in part by a Natural Sciences and Engineering Research Council of Canada Postgraduate grant and an Ontario Graduate Scholarship in Science and Technology.

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FIG. 1.

Isomorphic amplification of inhibitory postsynaptic currents (IPSCs) evoked by stimulation of the ipsilateral common peroneal nerve (stimulated at 50 Hz), in a medial gastrocnemius motoneuron. *A*: current injected by voltage clamp during steps to holding potentials of 10, 15, 20, 25, and 30 mV relative to the resting membrane potential. Raw recordings are in black; low-pass filtered recordings are in gray. Thick gray bars indicate the time of ipsilateral common peroneal nerve stimulation. *B*: injected current during the first 500 ms of the voltage-clamp steps. The voltage-clamp step that evoked the largest persistent inward currents (PICs) is shown in gray. *C*: current-voltage ($I-V$) relation with current measured as the time-average of the response during the first 500 ms of the voltage-clamp step (solid line) and linear extrapolation to current measured in response to voltage-clamp steps from -5 to 10 mV (dashed line). The linear fit was $y = 1.24x$. The largest PICs were activated by a step to 30 mV from rest (asterisk). *D*: IPSCs measured during voltage-clamp steps to holding potentials of 10, 15, 20, 25, and 30 mV from rest. *E*: relationship between the peak of the IPSCs and the holding potential. Note the large supralinear increase during the 30-mV voltage-clamp step.

FIG. 2.

Pleomorphic amplification of IPSCs evoked by stimulation of the ipsilateral medial gastrocnemius nerve (stimulated at 50 Hz_s) in a tibialis anterior motoneuron. *A*: current injected by voltage clamp during steps to holding potentials of 0, 5, 10, 15, and 20 mV relative to the resting membrane potential. Raw recordings are in black; low-pass filtered recordings are in gray. Thick gray bars indicate the time of ipsilateral medial gastrocnemius nerve stimulation. *B*: injected current during the first 500 ms of the voltage-clamp steps. The voltage-clamp step that evoked the largest PICs is shown in gray. *C*: I - V relation with current measured as the time-average of the response during the first 500 ms of the voltage-clamp step (solid line) and linear extrapolation to current measured in response to voltage-clamp steps from 0 to 10 mV (dashed line). The linear fit was $y = 1.10x + 0.40$. The largest PICs were activated by voltage-clamp step to 20 mV from rest (asterisk). *D*: IPSCs measured during voltage-clamp steps to holding potentials of 0, 5, 10, 15, and 20 mV from rest. *E*: relationship between the peak of the IPSCs and the holding potential. Note the supralinear increase in size during the 20-mV voltage-clamp step.

FIG. 3. Pleomorphic and isomorphic forms of IPSC amplification by PICs. *A*: IPSCs from Fig. 1*D* were normalized to the peak of the largest IPSC. *B*: IPSCs from Fig. 2*D* were normalized to the peak of the largest IPSC.

FIG. 4.

Change of IPSC time course by PICs. The change of the IPSC time course was measured as the increase in the plateau size (in relation to the peak) between the largest IPSC (termed “peak”) and the IPSCs measured during holding steps from -5 to 10 mV (termed “subthreshold”). Each data point represents the average increase (negative values indicate a decrease) in plateau size between the amplified IPSC and all the IPSCs. The error bars represent the SD. All IPSCs were normalized to the peak of the amplified IPSC before calculation of the ratio.

FIG. 5.

Amplification of peak of IPSC and of time integral of IPSC by PICs. The amplification of the peak of the IPSC by PICs (filled circle) was calculated as the ratio between the measured peak of the amplified IPSC and the predicted peak of the IPSC estimated using extrapolation of the peak of the IPSCs measured during holding steps from -5 to 10 mV. The amplification of the time integral of IPSC by PICs (open circle) was calculated similarly.

FIG. 6.

Simulations based on a compartmental model of a motoneuron with L-type Ca^{2+} channels distributed in $100\ \mu\text{m}$ hot spots centered 100 to $400\ \mu\text{m}$ away from the cell body and with inhibitory synapses distributed according to anatomical observations by Fyffe (1991). Three different values of the peak conductance of the inhibitory synapses, g_{peak} , were simulated. *Left column:* $g_{\text{peak}} = 3\ \text{nS}$. *Center column:* $g_{\text{peak}} = 4.5\ \text{nS}$. *Right column:* $g_{\text{peak}} = 6\ \text{nS}$. *A:* current reaching the cell body during the voltage-clamp steps to holding potentials of -5 to $30\ \text{mV}$ relative to the resting membrane potential in 5-mV increments. *Inset:* I - V relation with current measured as the time-average of the response during the first $500\ \text{ms}$ of the voltage-clamp step (solid line) and linear extrapolation to current measured in response to voltage-clamp steps from -5 to $10\ \text{mV}$ (dashed line). The linear fit was $y = 0.35x - 0.70$. *B:* IPSCs during the voltage-clamp steps. Arrows denote the largest IPSC in terms of the peak of the IPSC. *C:* IPSCs normalized to the peak of the largest IPSC.

FIG. 7.

Probability of the opening of channels in every hot spot in a motoneuron model during a voltage-clamp step to 20 mV, relative to the resting membrane potential. *A*: $g_{\text{peak}} = 3 \text{ nS}$. *B*: $g_{\text{peak}} = 6 \text{ nS}$. *C*: comparison of the IPSCs during the 20-mV step from rest with $g_{\text{peak}} = 3 \text{ nS}$ and $g_{\text{peak}} = 6 \text{ nS}$, normalized to the peak of the IPSC with $g_{\text{peak}} = 6 \text{ nS}$. *D*: percentage of hot spots activated as defined by a probability of channel opening >0.5 for $g_{\text{peak}} = 3 \text{ nS}$ and with $g_{\text{peak}} = 6 \text{ nS}$. *E*: maximal increase in plateau size with $g_{\text{peak}} = 6 \text{ nS}$ and different time constants of activation of the L-type Ca^{2+} channels.

FIG. 8.

Box plots of electrotonic properties of activated L-type Ca^{2+} channel hot spots that were either deactivated or not deactivated by inhibitory synaptic activity, where $g_{\text{peak}} = 6 \text{ nS}$. *Left:* number of inhibitory synapses within 0.1λ . *Middle:* electrotonic distance from the cell body. *Right:* local passive input resistance. The boundaries of the box indicate the 25th and 75th percentiles. Whiskers above and below the box indicate, respectively, the 90th and 10th percentiles. A line within the box represents the median.