Active Dendritic Integration of Inhibitory Synaptic Inputs In Vivo

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Kuo, Jason J., Robert H. Lee, Michael D. Johnson, Heather M. Heckman, and C. J. Heckman. Active dendritic integration of inhibitory synaptic inputs in vivo. J Neurophysiol 90: 3617–3624, 2003. Synaptic integration in vivo often involves activation of many afferent inputs whose firing patterns modulate over time. In spinal motoneurons, sustained excitatory inputs undergo enormous enhancement due to persistent inward currents (PICs) that are generated primarily in the dendrites and are dependent on monoaminergic neuromodulatory input from the brain stem to the spinal cord. We measured the interaction between dendritic PICs and inhibition generated by tonic electrical stimulation of nerves to antagonist muscles during voltage clamp in motoneurons in the lumbar spinal cord of the cat. Separate samples of cells were obtained for two different states of monoaminergic input: standard (provided by the decerebrate preparation, which has tonic activity in monoaminergic axons) and minimal (the chloralose anesthetized preparation, which lacks tonic monoaminergic input). In the standard state, steady inhibition that increased the input conductance of the motoneurons by an average of 38% reduced the PIC by 69%. The range of this reduction, from <10% to >100%, was proportional to the magnitude of the applied inhibition. Thus nearly linear integration of synaptic inhibition may occur in these highly active dendrites. In the minimal state, PICs were much smaller, being approximately equal to inhibition-suppressed PICs in the standard state. Inhibition did not further reduce these already small PICs. Overall, these results demonstrate that inhibition from local spinal circuits can oppose the facilitation of dendritic PICs by descending monoaminergic inputs. As a result, local inhibition may also suppress active dendritic integration of excitatory inputs.

INTRODUCTION

Voltage-sensitive currents in the dendrites of neurons are likely to play a major role in synaptic integration. Much of our present understanding of the effect of active dendrites on synaptic integration is based on studies of individual postsynaptic potentials (PSPs) (Hausser et al. 2000). However, a background of tonic synaptic activity is often present in vivo (Steriade 2001). Furthermore, much of normal information processing requires the genesis of firing rates that modulate over time. Good examples of this modulation of firing rate occur in the motor system, where firing rates of pyramidal neurons and spinal motoneurons are matched to the smooth progression of many movements (Ashe and Georgopoulos 1994; Holdefer and Miller 2002; Morrow and Miller 2003). Presumably, such firing patterns require integration of many PSPs undergoing both spatial and temporal summation.

In spinal motoneurons, where all motor commands are translated into outputs to muscle fibers, dendritic integration of sustained inputs is under the control of neuromodulatory inputs from the brain stem (Powers and Binder 2001). Perhaps the most important of these neuromodulators are the monoamines serotonin and norepinephrine. Motoneurons have the capacity to generate a large persistent inward current (PIC; Schwindt and Crill 1980), but this capacity can only be realized if monoamines are present (Hounsgaard and Kiehn 1985; Hounsgaard et al. 1988; Perrier and Hounsgaard 2003). Much of the PIC has been shown to originate in dendritic regions (Carlin et al. 2000b; Hounsgaard and Kiehn 1993; Lee and Heckman 1998a, 1999a, 2000; Svirsks and Hounsgaard 1998).

Bistable behavior, in which a brief excitatory input initiates long-lasting self-sustained firing, was the first effect of the PIC to be demonstrated in preparations with monoamines (Hounsgaard and Kiehn 1985; Hounsgaard et al. 1988). However, self-sustained firing in the absence of input only occurs in low threshold motoneurons that are likely to innervate slow twitch muscle fibers (Lee and Heckman 1998b). This does not limit the importance of the dendritic PIC to motor behaviors, such as posture, that are dominated by slow twitch motor units. In every motoneuron, low- or high-threshold, the dendritic PIC markedly alters the response to injected or synaptic currents while they are being applied to the cell.

The PIC imparts a strong acceleration in firing rate during application of injected current (Bennett et al. 1998, 2001; Hornby et al. 2002; Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988; Lee and Heckman 1998b). Excitatory synaptic inputs are enhanced to an even greater degree by the PIC—as much as fivefold (Bennett et al. 1998; Delgado-Lezama et al. 1999; Lee and Heckman 2000; Lee et al. 2003; Prather et al. 2001). Synaptic inhibition, while less studied, has been shown to turn off self-sustained firing (Hounsgaard et al. 1988) and to strongly influence the acceleration in firing rate generated by the PIC (Bennett et al. 1998). In this study, we quantify the interaction between the dendritic PIC and inhibitory synaptic input with single-electrode voltage clamp techniques. All studies were carried out in vivo, with an inhibitory input that was predominated by reciprocal inhibition between antagonist motor pools. Our hypothesis was that inhibition would reduce the
dendritic PIC, either by hyperpolarization and by shunting of the active currents (cf. Powers and Binder 2000) or by metabotropic actions (Svirskis and Hounsgaard 1998), resulting in a much more linear current-voltage relation. We found that the degree of reduction of the PIC was approximately linearly related to the amplitude of the inhibition, suggesting that synaptic integration of inhibitory inputs in motoneurons with highly active dendrites could be a linear process.

Methods

All animal procedures were fully approved by the Northwestern University animal care committee. Most experiments ($n = 8$) were performed in the decerebrate cat preparation. Details of initial surgical procedures are available in our previous work (Heckman et al. 1994; Lee and Heckman 1998b). Briefly, all surgical preparations of the spinal cord and hindlimb were done under deep gaseous anesthesia (1.5–3.0% isoflurane in a 3:1 mixture of $O_2$ and $N_2O$). The preparation included a laminectomy to expose the $L_1$ and $S_1$ segments of the spinal cord for intracellular recording. In the hindlimb, the nerves to the medial gastrocnemius (MG) and lateral gastrocnemius-soleus (LGS) muscles were isolated and placed on hook-shaped bipolar stimulating electrodes. The Achilles tendon was surgically isolated (the plantaris tendon was cut, leaving only the MG and LGS tendons) and attached to a computer-controlled muscle puller. The common peroneal nerve (CP), which contains nerves of several antagonists to MG and LGS, was isolated and placed in a cuff electrode. The gaseous anesthesia was discontinued after a precollicular decerebration. A bilateral pneumothorax was created to enhance artificial respiration. A bilateral pneumothorax was created to enhance artificial respiration. A bilateral pneumothorax was created to enhance artificial respiration. A bilateral pneumothorax was created to enhance artificial respiration.

Intracellular recordings of motoneurons were obtained in the lumbar cord with sharp microelectrodes and identified by antidromic stimulation of either the MG or LGS nerves. Microelectrode tips were broken back under microscopic observation and control. Because of the large currents required for successful single-electrode voltage clamp techniques in spinal motoneurons, resistances of the electrodes were kept low—typically around 3–4 MΩ in saline before entering the cord. In the initial studies in the decerebrate, eight cells were recorded with electrodes filled with a solution combining potassium citrate (1.5 M) and potassium chloride (1.5 M). Most data (19 cells) in the decerebrate and all data in the chloralose preparation (7 cells) were taken with electrodes filled with 2 M potassium citrate to avoid alteration of the reversal potential for the inhibition, which is sensitive to internal chloride concentration (Coombs et al. 1955). The inhibitory reversal potential was not systematically measured, but in two cells in the KCl sample, we noted an approximate 5-mV depolarization of the reversal potential during the 4–5 min required to obtain the measurements presented in this study. In the decerebrate preparation, there were no significant differences in the amplitude of the inhibition or its effect on the PIC in the two samples recorded with different electrode solutions ($t$-test, $P > 0.3$). In all cells, voltage clamp was applied using the single-electrode discontinuous mode (Axoclamp 2A amplifier, Axon Instruments; switching frequency of 8–10 kHz; data with inadequate settling of electrode transients were rejected).

Levels of monoaminergic input to the cord

The main difference between the two preparations described above is that the decerebrate has tonic activity in the monoaminergic axons originating in the brain stem (Baldissera et al. 1981; Lee and Heckman 1998b). All cells recorded in the decerebrate preparation were thus considered to have a moderate level of monoaminergic input. Anesthesia reduces the PIC (Lee and Heckman 2000), presumably through a reduction in activity of monoaminergic axons. Therefore the cells recorded in the chloralose preparation were considered to have a low level of monoaminergic input. To be consistent with our previous work on PIC effects on excitatory synaptic input (Lee and Heckman 2000), we refer to the decerebrate preparation as providing the “standard” state for monoaminergic input to the cord and the deeply anesthetized preparation as the “minimal” state. The “enhanced” state of the previous study, which depended on the addition of a noradrenergic agonist, was not employed in the present work.

Intracellular recordings

Intracellular recordings of motoneurons were obtained in the lumbar cord with sharp microelectrodes and identified by antidromic stimulation of either the MG or LGS nerves. Microelectrode tips were broken back under microscopic observation and control. Because of the large currents required for successful single-electrode voltage clamp techniques in spinal motoneurons, resistances of the electrodes were kept low—typically around 3–4 MΩ in saline before entering the cord. In the initial studies in the decerebrate, eight cells were recorded with electrodes filled with a solution combining potassium citrate (1.5 M) and potassium chloride (1.5 M). Most data (19 cells) in the decerebrate and all data in the chloralose preparation (7 cells) were taken with electrodes filled with 2 M potassium citrate to avoid alteration of the reversal potential for the inhibition, which is sensitive to internal chloride concentration (Coombs et al. 1955). The inhibitory reversal potential was not systematically measured, but in two cells in the KCl sample, we noted an approximate 5-mV depolarization of the reversal potential during the 4–5 min required to obtain the measurements presented in this study. In the decerebrate preparation, there were no significant differences in the amplitude of the inhibition or its effect on the PIC in the two samples recorded with different electrode solutions ($t$-test, $P > 0.3$). In all cells, voltage clamp was applied using the single-electrode discontinuous mode (Axoclamp 2A amplifier, Axon Instruments; switching frequency of 8–10 kHz; data with inadequate settling of electrode transients were rejected). Low-frequency gain of the feedback loop (−3 dB of 30 Hz) was enhanced 11-fold by an external circuit, resulting in gains that ranged from about 100 to 300 nA/mV (details in Heckman and Lee 2001; Lee and Heckman 1998a).

Generation of synaptic input

Inhibition of MG and LGS motoneurons was generated by 100-Hz stimulation of the CP nerve. The intensity of the stimulation of the CP nerve was varied in different experiments to allow investigation of different levels of inhibition. Intensities in most experiments stayed within the group I range (1.2–1.8 times motor threshold; assessed before paralysis). In one experiment, intensity was increased to 2.4 times threshold. Repeated trials of different intensities within a single cell were not possible because the PIC tended to decay drastically with more than three or four repeats of the I-V function. The CP innervates all the muscles in the anterior compartment of the lower leg as well as on the dorsum of the foot and also includes cutaneous afferents from the dorsum of the foot. However, the low threshold of the stimulation in most experiments likely restricted the input to group I muscle afferents of antagonist muscles and was probably dominated by Ia reciprocal inhibition (see Discussion for possible roles of other afferents). As in our previous study with reciprocal inhibition (Heckman and Binder 1991a), a modest peak in inhibitory effective synaptic current occurred in the first 0.2 s followed by decay to about 70–80% of this peak amplitude by 0.5–0.6 s. Thereafter, the value decayed by no more than 10–15% for the next 3–4 s. Rate of decay after the first 3–4 s was not systematically studied. However, in about one-half the cells, the inhibition underwent a significant decline 5–6 s after stimulus onset. Consequently, all measurements of inhibition on the PIC were confined to the first 3–4 s.

Experimental protocols

A slow (12–16 mV/s) ramp-shaped voltage command was applied to define the current-voltage (I-V) relationship of the cell (see Fig. 1). Voltage returned to baseline at the same rate, but our analysis focused only on the depolarizing or “up” portion of the ramp to minimize the effect of the time-dependent decay in the synaptic inhibition noted above. We have previously shown that this slow rate of change of voltage provides a good estimate of the cell’s steady-state I-V function (Lee and Heckman 1998a). Precisely the same ramp-shaped voltage command was then repeated during a steady background of inhibition, with the most depolarized level for the I-V function being reached within 3 s (Fig. 1). This I-V function plus inhibition was followed by
RESULTS

Inhibition suppresses the dendritic PIC in the standard state of monoaminergic input

As in our previous studies (Lee and Heckman 1998a, 1999a,b, 2000), the I-V functions in motoneurons in this study exhibited a moderately strong PIC in the preparation with the standard state of monoaminergic input to the cord (i.e., the decerebrate; see METHODS). This PIC was usually evident as a strong downward deflection in the current beginning at about −50 mV, as shown by the example in Fig. 1A (trace labeled “control current”). The same voltage command was repeated during a steady background of inhibition (see METHODS). Because of the high-voltage clamp feedback achieved with our techniques (see METHODS), the addition of inhibition caused virtually no change in the voltage clamp control of the region of the cell near the electrode (which was presumed to be in the soma). Actual voltage traces for the currents with and without inhibition are shown in Fig. 1A. There was never more than a 0.5-mV difference at any point along the voltage ramps either with or without inhibition in any of the cells (in this example the maximum difference was 0.3 mV).

If the inhibitory effects were ionotropic (see DISCUSSION) and were confined to the region of the cell under good clamp control, the inhibition would have simply added an offset and an increase in slope to the control current. This is indeed what occurred at hyperpolarized levels (e.g., −60 to −70 mV; see trace labeled “Current during inhibition” in Fig. 1A). However, at more depolarized levels, the added inhibition nearly eliminated the PIC. Figure 2A shows the results for Fig. 1A with the leak subtracted (see METHODS) and current plotted versus voltage. In control conditions, the amplitude of the PIC reached nearly 25 nA (solid trace). The inhibition reduced the PIC to only about 4 nA (dashed trace). This large reduction in PIC amplitude occurred either by ionotropic actions in portions of the cell outside the region of good clamp control (i.e., in the dendrites) or via metabotropic actions (see DISCUSSION).

We successfully obtained data similar to that shown in Figs. 1A and 2A in 20 cells in the standard monoaminergic state. In this sample, the steady background of inhibition increased the input conductance of the cell by an average of approximately 38%, from 1.10 ± 0.29 to 1.57 ± 0.29 (SD) μS (this difference was statistically significant, with \( P < 0.0001 \) using a paired \( t \)-test). Figure 3A illustrates that the change in input conductance due to inhibition ranged widely across this sample of cells, allowing us to analyze the relation between the amplitude of the inhibition and its effect on the PIC (see Scaling of PIC amplitude by inhibition).

Inhibition had a strong impact on the amplitude of the PIC. On average, the inhibition reduced the PIC by approximately 69%, from 18.8 ± 7.7 to 5.9 ± 8.7 nA (this difference was significant; \( P < 0.0001 \); paired \( t \)-test). Figure 3B shows that there was considerable variation about this average change, ranging from virtually no effect to three cells in which the PIC was not only reduced but was converted to a net outward current (see the three data points below the zero line). An example of a cell in which the inhibition produced a net outward current is shown in Fig. 2B. The scatter in Fig. 3, A and B, did not appear to be due to inter-animal variability, because the scatter within one particularly successful experiment (symbols identified by diamonds with crosses) was sim-
ilar to that for the whole sample. Neither the amplitude of the PIC in the control state nor the minimal state were significantly correlated with input conductance ($P > 0.3$), indicating that there were no differences in inhibitory suppression of PIC in motoneurons innervating slow and fast muscle units. The voltage for the peak amplitude of the PIC did not differ with and without inhibition (paired $t$-test, $P > 0.3$), indicating that, unlike the case for excitatory input (Lee and Heckman 2000), the inhibitory effect on the PIC was not accompanied by a shift in the range of PIC activation. This lack of effect of inhibition on PIC activation voltage may imply a metabotropic action for inhibition on the PIC (see Discussion).

**Dendritic PICs in the minimal state are small and unaffected by inhibition**

In the minimal monoaminergic state (in the chloralose anesthetized preparation; see Methods), an entirely different result was obtained. In the example shown in Fig. 1B, there was very little PIC in the control condition and the inhibitory background acted mainly to increase the offset and slope of the control current. Figure 2C shows the leak-subtracted PICs for control (thin solid trace) and inhibitory conditions (thin dashed line) for the same cell as Fig. 1B. The PIC was small in the control case and the inhibition had little further impact.

In the minimal state, results for seven cells showed that inhibition increased input conductance by approximately 21%, which was not significantly different from the increase in the standard preparation ($t$-test, $P > 0.3$; see the triangular symbols in Fig. 3A). Consistent with our previous work (Lee and Heckman 2000), the average control PIC amplitude in the minimal state ($3.4 \pm 4.2$ nA) was only about 20% as large as the PIC amplitude in the standard preparation (compare x axis of Fig. 3A).

**FIG. 2.** Current-voltage relationships for the data shown in Fig. 1. Sub-threshold leak has been subtracted (see Methods). A: standard monoaminergic state. The PIC is large during control conditions (solid line) but greatly reduced during inhibition (dashed line), even though voltage ramps were virtually identical (see Fig. 1). B: standard monoaminergic state. PIC in this cell was converted to a net outward current. C: minimal monoaminergic state. Control PIC is much smaller than in the standard state, and inhibition has little further effect.

**FIG. 3.** Inhibition induced changes in input conductance and PIC amplitude. In both parts of the figure, diamonds indicate data from the standard monoaminergic state and triangles from the minimal state. Crosses through some of the diamonds indicate data from a single experiment. A: change in input conductance induced by the inhibition (the difference between input conductance with and without a steady background of inhibition) is plotted vs. the control input resistance. B: PIC amplitude during a steady background of inhibition plotted vs. control PIC amplitude. Solid lines and corresponding equations are the regression relationships for the data in the standard monoaminergic state. Dashed line has a slope of 1.0 and a y axis intercept of 0, indicating no effect of inhibition on the PIC.
values for triangles and diamonds in Fig. 3B). Unlike the case for the standard state, inhibition in the minimal state did not reduce PIC amplitude compared with its control value (3.2 ± 2.4 nA for inhibition, *P* > 0.8, paired *t*-test).

Note that the average amplitudes of the control PIC in the minimal state (3.4 nA) and the PIC with inhibition in the standard state (5.9 nA) were not significantly different (*t*-test, *P* > 0.3). Thus inhibition can have nearly as powerful an effect on PIC amplitude as anesthesia, which acts to suppress monoaminergic input to the cord. In one important respect, the effect of inhibition was stronger than anesthesia because, as noted above, the inhibition converted the PIC in the standard state to a net outward current in several of the standard state cells (again, see the 3 cells below the 0 line in Fig. 3B), whereas the anesthesia in the minimal state produced small but positive PIC amplitudes (triangles in Fig. 3B).

**Inhibitory effective synaptic current is enhanced by the PIC**

The large suppression of the PIC in the standard preparation constitutes a considerable enhancement in the efficacy of the inhibitory input. This enhancement can be appreciated from measurements of the inhibitory effective synaptic current \( I_N \); i.e., the net inhibitory current reaching the soma (Lee and Heckman 2000), which includes the effect of the dendritic PIC. The inhibitory \( I_N \) is the difference between the cell’s *I-V* function with and without the steady inhibitory background, i.e., the difference between the two current traces in Fig. 1, A and B. The inhibitory \( I_{NS} \) for the data in Fig. 1, A and B, are plotted as a function of voltage in Fig. 4. In the cell in the standard preparation (thick trace), the inhibitory \( I_N \) undergoes a marked increase at about −45 mV. This enhancement is due to the inhibitory suppression of the PIC. In contrast, in the minimal preparation (thin trace), inhibition smoothly increases with depolarization but lacks the enhancement due to PIC suppression. On average, the peak inhibitory \( I_N \) in the standard preparation was −24.6 ± 14.5 nA. The inhibitory \( I_N \) in the minimal preparation usually lacked a clear peak, as in the example in Fig. 4. Its “peak” value was therefore measured at the same voltage as the peak of the leak-subtracted PIC, giving an average value of −8.6 ± 4.0 nA. This nearly threefold average difference in inhibitory \( I_N \) between the minimal and standard states was statistically significant (*P* < 0.001, *t*-test).

**Scaling of PIC amplitude by inhibition**

The peak amplitude of the inhibitory \( I_N \) for the standard state had a nearly 10-fold range of values (−5.6 to −55.0 nA). The reduction in amplitude of the PIC due to inhibition (i.e., the difference in PIC amplitude with and without the background of inhibition) had a similarly wide range (−0.9 to −28.6 nA). One possibility is that inhibitory \( I_N \) and the suppression of the PIC simply scaled proportionally with the magnitude of the inhibition applied to each cell. This magnitude was assessed from the change in input conductance generated by the inhibition (see Fig. 3A; note that this measurement is taken at a hyperpolarized levels to avoid the effects of the PIC). Figure 5A shows that peak inhibitory \( I_N \) was strongly correlated (\( r = −0.86, P < 0.001 \)) to inhibition magnitude. In part this correlation is to be expected: the greater the inhibition-induced change in slope of the *I-V* function, the greater the inhibitory current at depolarized levels where \( I_N \) is assessed (synaptic current being equal to driving force times conductance).
change). Therefore we also assessed the relation between the inhibition-induced reduction in PIC amplitude and inhibition magnitude. The resulting correlation was moderate ($r = -0.66, P < 0.001$). However, the reduction in PIC amplitude was also significantly correlated with the amplitude of the control PIC ($r = 0.53, P < 0.01$). Thus we normalized the reduction in PIC amplitude by dividing it by the control PIC amplitude. Figure 5B shows the relation between the normalized reduction in PIC amplitude and inhibition magnitude. The three cells in which the inhibition converted the PIC to a net outward current (circles) were clear and obvious outliers and were excluded from the regression analysis, resulting in a strong correlation ($r = -0.81$). As inhibition magnitude increased, the PIC was reduced to near zero amplitude (the line at $-1.0$ indicates where PIC amplitude was 0). The results of Figs 5, A and B, suggest that inhibition linearly scales the PIC, so that integration of inhibitory input in motoneurons with highly active dendrites may be a linear process. Figure 5B also suggests that cells with strong outward dendritic currents may behave very differently than cells where the PIC dominates.

**DISCUSSION**

Motoneurons studied in preparations with significant levels of the monoamines serotonin or norepinephrine exhibit strong PICs that have a primarily dendritic origin (Carlin et al. 2000a,b; Lee and Heckman 1998a, 1999a; Svärsksis and Hounsgaard 1998). These dendritic PICs have previously been shown to enhance ionotropic excitatory synaptic input by as much as much as five- to sixfold, depending on the level of monoaminergic input (Lee and Heckman 2000; Lee et al. 2003). The results of this study showed that a steady background of synaptic inhibition markedly suppressed the dendritic PIC. In fact, the effect of inhibitory suppression on the dendritic PIC was as strong as that of anesthesia (which likely suppresses the monoaminergic drive to the spinal cord). Because the dendritic PIC has a major impact on the net input-output gain of the motoneuron, our results suggest that inhibition can be used to counterbalance descending monoaminergic input in controlling the gain of the motoneuron pool. The linear relation between magnitude of the applied inhibition and the reduction in PIC amplitude suggest that integration of inhibitory input to motoneurons with strong PICs may be remarkably linear.

**Ionotropic versus metabotropic inhibitory effects on the dendritic PIC**

The inhibition used in this study was generated by electrical stimulation of a nerve that innervates muscles that are antagonists to those innervated by the motoneurons studied. Because the stimulation intensity was low (usually well within the group I range), this input was likely to have been dominated by Ia afferents acting via Ia inhibitory interneurons. However, there may have been a modest contribution from other pathways as well. Ia afferents connect to nonreciprocal group I interneurons that can inhibit extensor motoneurons (Jankowska 1992). Furthermore, the common peroneal nerve has cutaneous and joint afferents as well as muscle afferents. Our stimulation could have also activated very low threshold cutaneous afferents with possible inhibitory or excitatory actions. However, any excitation via this source must have been small, as inhibition clearly dominated our results. The relative contribution of the ionotropic inhibitory receptors (glycine and GABA$_A$) compared with metabotropic inhibitory receptors (GABA$_B$) for these various inputs is not clear. Reciprocal inhibition has been considered a classic example of a glycinergic input to motoneurons (Jankowska 1992). However, synaptic boutons in the ventral cord co-release glycine and GABA (Jonas et al. 1998) and glycine and GABA$_A$ receptors are co-localized on motoneurons (Fyffe 2001; Geiman et al. 2002; Todd et al. 1996). Consequently, the relative importance of glycine and GABA in reciprocal inhibition is uncertain at present (Fyffe 2001). The key question for interpretation of the inhibitory suppression of the PIC is whether any of these inputs produced significant activation of the postsynaptic GABA$_B$ receptors. Curtis and Lacey (1998) suggested that inhibitory actions of the GABA$_B$ agonist baclofen on monosynaptic reflexes may not be presynaptic (see also Stuart and Redman 1992), but instead occur via a postsynaptic, dendritic location. Baclofen has been shown to suppress the motoneuron PIC in both turtle (Svärsksis and Hounsgaard 1998) and rat motoneurons (D. Bennett, personal communication) as well as in spinal interneurons (Drojean et al. 2003). If GABA was released by any of the interneurons activated by the common peroneal nerve, the high-frequency (100 Hz) and long duration of the stimulation could result in significant spillover of GABA onto GABA$_A$ receptors.

Thus both ionotropic and metabotropic actions are possible. Our results appear to suggest involvement of both mechanisms. Inhibitory input invariably increased the input conductance of the cell, which presumably reflects the opening of ion channels. However, the lack of effect of inhibition on the voltage at which the peak of the PIC occurred is not consistent with an ionotropic mechanism. A hyperpolarization of unclamped dendrites would be expected to shift this voltage in a depolarized direction during the voltage ramp applied the soma, just as the depolarization of the dendrites by ionotropic excitatory input shifts PIC activation during the voltage ramp at the soma to a more hyperpolarized level (Lee and Heckman 2000). In contrast, changing PIC amplitude without altering its voltage range is exactly what happens when the level of monoaminergic input to the cord is altered (Lee and Heckman 1999a). Thus we have a paradoxical result: a subthreshold, apparently ionotropic effect (change in input conductance), provides an excellent prediction of a suprathreshold, apparently metabotropic effect (suppression of the PIC). Further work with more specific inhibitory inputs (e.g., recurrent inhibition) and with antagonists for metabotropic receptors are needed to help resolve this issue. An interesting question in this regard is the location of the inhibition, proximal or distal. For example, recurrent inhibition from Renshaw cells is mediated by more distal synapses than is reciprocal inhibition (Fyffe 2001). Recurrent inhibition appears to be highly effective in suppressing the acceleration in firing rate generated by activation of the PIC (H. Hultborn, personal communication).

An interesting point was that the PIC was converted to a net outward current in three cells. The reason why this conversion to outward current occurred in these three cells compared with others was not clear, because these three cells were not unusual with respect to their other parameters (magnitudes of inhibition and amplitudes of PICs were within the midrange of values for all cells). Nonetheless, these results make it clear that the balance of inward and outward currents in the dendrites of
motoneurons is likely to have a major impact on synaptic integration.

**Control of PIC amplitude**

Although the relative roles of ionotropic and metabotropic receptors are uncertain, it is very clear that the inhibition from the common peroneal nerve was very effective in reducing the PIC. On average, the inhibition was as potent as anesthesia in suppressing the PIC. When strong enough, the inhibition can reduce the PIC to zero (Fig. 5B), suggesting that inhibition by local spinal circuits can be used to counterbalance the facilitatory actions of descending monoaminergic inputs on PICs. Our results are consistent with those of Bennett et al. (1998), showing that inhibition increased the frequency and current at which PIC-induced acceleration in firing occurred. This increase might simply reflect a smaller PIC, so that the voltage at which PIC becomes large enough to generate an acceleration in firing is depolarized. Overall, inhibitory actions on the PIC should have a strong influence on the gain of motor outflow because the amplitude of the PIC has a large influence on the net input-output gain of the motoneuron (see Lee et al. 2003) and thus also on the gain of the entire motor pool (see Heckman 1994; Heckman and Binder 1991b). This effect of inhibition on gain is in direct contrast to the classical picture of excitative and inhibitory interactions, which simply produce net shifts in motoneuron frequency-current relations but do not alter the slope (i.e., gain) (reviewed in Powers and Binder 2001). It should be noted, however, that PIC-dependent gain changes likely only apply to inputs with physiological rates of change (e.g., time courses of 50–100 ms or longer). Transient inputs such as the H-reflex generated by single electrical shocks may not induce strong activation of the PIC. Overall, two points emerge from the present results on inhibition and the previous studies of excitatory inputs (Bennett et al. 1998; Delgado-Lezama et al. 1999; Lee and Heckman 2000; Lee et al. 2003; Prather et al. 2001). The dendritic PIC totally dominates synaptic integration for excitatory inputs and the degree of this effect can be controlled both by descending monoaminergic input and by local inhibitory input within the spinal cord.

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**DISCLOSURES**

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