Spinal Cord Injury Causes Plasticity in a Subpopulation of Lamina I GABAergic Interneurons

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Submitted 4 October 2007; accepted in final form 8 May 2008

Dougherty KJ, Hochman S. Spinal cord injury causes plasticity in a subpopulation of lamina I GABAergic interneurons. J Neurophysiol 100: 212–223, 2008. First published May 14, 2008; doi:10.1152/jn.01104.2007. Dysfunction of the spinal GABAergic system has been implicated in pain syndromes following spinal cord injury (SCI). Since lamina I is involved in nociceptive and thermal signaling, we characterized the effects of chronic SCI on the cellular properties of its GABAergic neurons fluorescently identified in spinal slices from GAD67-GFP transgenic mice. Whole cell recordings were obtained from the lumbar cord of 13- to 17-day-old mice, including those having had a thoracic segment (T8,11) removed 6–9 days prior to experiments. Following chronic SCI, the distribution, incidence, and firing classes of GFP+ cells remained similar to controls, and there were minimal changes in membrane properties in cells that responded to current injection with a single spike. In contrast, cells displaying tonic/burst firing had more depolarized membrane potentials, increased steady-state outward currents, and increased spike heights. Moreover, higher firing frequencies and spontaneous plateau potentials were much more prevalent after chronic SCI, and these changes occurred predominantly in cells displaying a tonic firing pattern. Persistent inward currents (PICs) were observed in a similar fraction of cells from spinal transects and may have contributed to these plateaus. Persistent Na+ and L-type Ca2+ channels likely contributed to the currents as both were identified pharmacologically. In conclusion, chronic SCI induces a plastic response in a subpopulation of lamina I GABAergic interneurons. Alterations are directed toward amplifying neuronal responsiveness. How these changes alter spinal sensory integration and whether they contribute to sensory dysfunction remains to be elucidated.

INTRODUCTION

The most superficial layer of gray matter, lamina I, is a spinal sensory relay site. Nociceptive and thermoreceptive input enters via Aδ and C fiber afferents and is transmitted to the thalamus, periaqueductal gray, and parabrachial area via lamina I ascending tract neurons (Craig 2000). GABAergic neurons constitute ~25% of the neuronal population of lamina I (Polgar et al. 2003; Todd and Spike 1993). GABAergic neurons in this region are at a nodal point in the control of pain processing and have been shown to play an important role in spinal cord function and dysfunction, particularly in relation to pain (Coull et al. 2003) and spinal cord injury (Craig 2002; Finnerup and Jensen 2004). Because descending modulatory inputs to the dorsal horn are predominantly inhibitory (Gehart et al. 2004; Millan 2002), their loss after spinal cord transection likely contributes to hyperexcitability in spinal circuits that can manifest in increased nociceptive reflexes (Suzuki et al. 2004). Resulting hyperexcitability of dorsal horn neurons causes chronic pain (Malan et al. 2002; Millan 1999; Zeilhofer 2005). GABAergic neurons in lamina I are in a key position for the control of pain transmission. However, the ways in which these cells are affected by chronic spinal cord injury is unknown.

Voltage-gated ion channels, including Na+, L-type Ca2+ (Ca2+), and T-type Ca2+ (Ca3) have been linked to spinal hyperexcitability in both injury and pain (Hains et al. 2003a; Heineke et al. 2004a). Most of these same channels have also been implicated in persistent inward currents (PICs) and plateau potentials in both motoneurons and dorsal horn neurons (Hains et al. 2003a; Heckman et al. 2003; Honsgaard and Kiehn 1989; Morisset and Nagy 1999). Where most studies have been carried out in motoneurons, plateau potentials have been reported in small subpopulations of neurons in the substantia gelatinosa (Yoshimura and Jessell 1989) and deep dorsal horn (Derjean et al. 2003; Monteiro et al. 2006; Morriset and Nagy 1999; Russo and Honsgaard 1996).

PICs with Na+ and/or Ca2+ components have been reported in a larger fraction of lamina I (Prescott and De Koninck 2005) and laminae II–IV neurons (Murase et al. 1986). PICs, particularly in the dorsal horn, are modulated by a number of intrinsic spinal and descending supraspinal transmitters including substance P, GABA, glutamate, 5-HT, and acetylcholine (Derjean et al. 2003; Herrero et al. 2000; Murase et al. 1986; Russo et al. 1997, 1998). PICs generating plateaus become more readily activated and more sensitive to modulation in motoneurons in the chronically transected cord (Harvey et al. 2006; Li and Bennett 2003), but the actions of PICs in dorsal horn neurons after chronic cord transection remain unstudied.

Recently, the GAD67-GFP transgenic mouse has proven useful in examinations of spinal GABAergic neurons (Dougherty et al. 2005; Heineke et al. 2004b). We have shown that GAD67-GFP identified lamina I GABAergic neurons divide into at least two different populations of neurons based on their firing properties (Dougherty et al. 2005). Given the importance of GABAergic neurons to the inhibitory control of excess spinal cord excitability after SCI, the main objective of the present study was to determine the effects of complete spinal cord transection on lamina I GABAergic membrane properties, focusing on changes in active cellular properties.

Some results have been presented in abstract form (Dougherty and Hochman 2005, 2006).

METHODS

All experimental procedures complied with the National Institutes of Health guidelines for animal care and the Emory Institutional Biomedical Research Bldg., Rm. 644, Emory University School of Medicine, 615 Michael St., Atlanta GA 30322 (E-mail: shochn2@emory.edu).

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Animal Care and Use Committee. Homozygotic GAD67-GFP mice obtained from Jackson Laboratory (Bar Harbor, ME) were used in all experiments. Lamina I was identified between the dorsal white matter and the relatively translucent substantia gelatinosa. No cell >20 μm from the edge of the white matter was considered (Chery et al. 2000). Some lamina I neurons may have been excluded from consideration because lamina I is thicker in the central part of the cord.

**Spinal cord transection**

Mice, postnatal day (P) 6–8, were anesthetized with 10% urethan (1.5 mg/kg). Following dorsal laminectomy to expose a segment of the thoracic spinal cord, one segment between T8 and T11 was removed. Gel foam was used to maintain the separation between rostral and caudal parts of the cord. Mice recovered for 6–9 days (mean = 8) before cell counting or electrophysiology experiments at P13–17 (mean = 15).

**Surface area and cell counts**

Mice at postnatal day (P) 14 were anesthetized with urethane (2 mg/kg ip) and perfused with 1:3 volume/body weight ice-cold 0.9% NaCl-0.1% NaNO, 1 unit/ml heparin, followed by equal volume/body weight of 4% paraformaldehyde or modified Laka’s fixative (4% paraformaldehyde, 0.2% picric acid, 0.16 M PO₃); pH 6.9. All spinal cords were isolated and postfixed 1 h, cryoprotected in 10% sucrose, 0.1 M PO₃, pH 7.4 until sectioned in 10-μm-thick slices on a cryostat (Leitz 1720).

Ten nonconsecutive sections (100 μm apart) from lumbar segments 1–3 of three control mice and three chronic SCI mice were used for comparison of surface area and lamina I cell numbers using the Neuro lucida image analysis system (MicroBrightField, Williston, VT). All cell counts can only be regarded as estimates since stereological techniques were not used. This should not affect comparisons between treatment groups.

**Electrophysiology**

Control and chronic SCI mice (P13–17) were anesthetized with urethan (2 mg/kg ip) and decapitated, and the spinal cord was carefully dissected out of the body cavity and placed in a cooled (≤4°C) artificial cerebrospinal fluid (ACSF) containing (in mM) 250 sucrose, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 25 glucose, 1.25 NaH₂PO₄, and 26 NaHCO₃ at a pH of 7.4. The ACSF was continuously oxygenated (Leitz 1720).

The ACSF was continuously oxygenated with 95% O₂-5% CO₂. Transverse (150–250 μm) spinal slices were cut from lumbar cord using a vibrating blade microtome (Leica VT1000 S). Slices were left to recover at room temperature for ≥1 h prior to the onset of experimentation. The slicing procedure was common to both groups and represents an acute spinal injury. Because an acute injury is implicit to these procedures, for simplicity we refer to the acutely injured group as the controls and to the mice with a chronic spinal transection followed by the acute injury as the SCI or spinal cord transected mice.

Patch electrodes were prepared from 1.5 mm OD capillary tubes (World Precision Instruments, Sarasota, FL) using a two-stage puller (Narishige PP83) to produce resistance values ranging from 5 to 8 MΩ. For the majority of the experiments, the intracellular recording solution contained (in mM) 140 K-gluconate or KF, 11 EGTA, 10 HEPES, 1 CaCl₂, and 26 NaHCO₃ (Leitz 1720). Some lamina I neurons may have been excluded from consideration because lamina I is thicker in the central part of the cord.

Some lamina I neurons were identified using epifluorescent illumination. Position of the cell in lamina I was verified using differential-interference contrast optics (DIC) at ×40 to show that the cell was in or adjacent to the white matter in that focal plane (Chen and Gu 2005). Then the electrode was lowered into the slice and the cell targeted for whole cell patch-clamp recordings using DIC. Both voltage- and current-clamp data were acquired on computer with the pCLAMP acquisition software Clampex (v 9.2; Molecular Devices; Union City, CA).

Immediately following rupture of the cell membrane (in voltage clamp at ~80 mV), the current-clamp recording configuration was used to determine resting membrane potential (except when CsF electrodes were used). Series resistance was subtracted. Experiments were conducted in both current- and voltage-clamp modes. In current clamp, cells were brought to ~80 mV by injecting bias current through the headstage. Then a series of 1-s hyperpolarizing and depolarizing current steps were undertaken. Liquid junction potentials were not corrected for. Firing type was determined by the response to current steps at and above threshold (Prescott and De Koninck 1996; Rusciewey and Sandkühler 2002). Membrane properties were measured and calculated as previously described (Dougherty et al. 2005). Peak inward currents were the maximum negative values measured during the first 20 ms of 100-ms voltage steps (~130 to +70 mV) from a holding potential of ~80 mV. Outward currents were measured as the average current during the last 20 ms of the highest voltage step in the same protocol. Voltage ramps (100 mV, 10 or 20 mVs) starting at −110 mV were used to examine PICs.

In some experiments, channel blockers were applied using a local perfusion system (SF-77B Perfusion Fast-Step; Warner Instrument, Hamden, CT). The following channel blockers were used (all from Sigma unless stated otherwise): tetrodotoxin (TTX; voltage-gated Na⁺; 1 μM), riluzole (persistent Na⁺; 10 μM; Tocris), CdCl₂ (non-specific Ca²⁺; 400 μM), NiCl₂ (T-type Ca²⁺; 100 μM), verapamil (L-type Ca²⁺; 50 μM), nifedipine (L-type Ca²⁺; 50 μM). Leak subtraction was performed on all current responses to voltage ramps using Clampfit (Kuo et al. 2005). Resistance was measured from the initial portion of the response where the slope was constant (1st 1.0–1.3 s).

A total of 162 lamina I GFP⁺ cells from 57 control mice and 90 cells from 23 SCI mice with resting potentials more negative than ~−40 mV were included in the analysis. However, for comparison of cell membrane properties other than resting potentials, only cells with a membrane potential more negative than ~−50 mV were considered. Statistical comparisons between firing properties were made using a Student’s t-test and reported as means ± SE. When distributions were not normal, a Mann-Whitney test was used. When distributions had unequal SDs, a Welch correction was used (InStat, GraphPad Software, San Diego, CA).

**RESULTS**

**Behavior and white/gray matter appearance**

Comparisons of thoracic spinal cord transections performed in neonate, weaning, and adult rodents have been previously described in great detail (Stelzner et al. 1975, 1979). Consistent with these reports, we observed hyperreflexive responses to develop in all mice within the first 5 postoperative days and weight support in some mice 7–8 days after spinal cord transection. Some mice did show stepping motions with their hindlimbs, particularly when moving over cage bedding (Guer-
There were no obvious correlations between behavior at the time of the experiment and results from cell recordings.

By 1 wk following spinal cord transection, white matter tracts were noticeably reduced (Fig. 1, A and B). Overall there was not a significant change in the surface area of the gray matter following spinal transection. However, measured ratios of white matter area to whole cord area demonstrated that white matter tracts had significantly degenerated in the SCI mice and constituted a smaller proportion of the cord area (32 ± 2%) after the week post injury as compared with the controls (42 ± 2%; \( P < 0.05 \)) (cf. Anelli et al. 2007).

Counts of GFP\(^{+}\) cells in lamina I demonstrated no difference in the number of GABAergic neurons between control (mean = 13 ± 1 per 10-\(\mu\)m section) and SCI mice (mean = 11 ± 2 per 10-\(\mu\)m section).

Incidence of firing properties

In cells from both control and SCI mice, three firing properties were seen in GFP\(^{+}\) lamina I neurons – tonic, initial burst, and single spike (Dougherty et al. 2005). The incidence of firing properties encountered were not statistically different between cells from control and spinal transected mice (\( P = 0.45 \) Fisher’s exact test). Toniaclly firing cells made up 34% of the control population and 42% of the population from SCI mice. Initial burst cells were encountered in 28 and 27% of cells from control and SCI mice, respectively. Thirty-four percent of control cells and 26% of cells from SCI mice were single spike. The remaining cells were unclassified (4% control, 6% SCI).

Cellular properties

Resting membrane potentials were compared between the control and SCI population (Fig. 2). When binned in 5-mV intervals and plotted as percent incidence, cells from both control and spinal transected mice show apparent bimodal distributions. The more depolarized peak is at −40 mV in both. However, the more hyperpolarized peak is 5 mV more hyperpolarized in cells from controls than in cells from SCI mice, suggesting that a subpopulation of neurons have undergone a depolarizing shift in their resting membrane potential. Shifting the control peak by 5 mV toward 0 aligns the distributions (Fig. 2, dashed line).

There were no differences in resistance, membrane time constant, cell capacitance, rheobase, and voltage threshold between the control and SCI cell populations. However, significant changes in membrane potential, steady-state outward currents and spike heights were observed (Table 1). Membrane potentials were more depolarized in cells from SCI mice (−59 mV) as compared with controls (−63 mV). Cells from SCI mice also had larger spikes (59 vs. 53 mV) and steady-state outward currents (1,170 vs. 1,475 pA).

Correlations between various membrane properties were compared (see Dougherty et al. 2005). For any two measured membrane properties, there were no overall differences in regression coefficients between cells from control and SCI mice or any evidence of changed distributions in neuron subpopulations based on firing type (data not shown). For example, in cells from control and SCI mice there was the same positive correlation between input resistance and membrane time constant (\( r = 0.56 \) in control and \( r = 0.59 \) in SCI mice), negative correlation between rheobase and membrane time constant (\( r = -0.72 \) and \( r = -0.70 \), in control and SCI mice, respectively), and lack of correlation between resistance and membrane potential (\( r = 0.15 \) in control, \( r = 0.11 \) in SCI mice).

Spinal transection-induced changes based on firing type

Single spike cells were unchanged in all properties measured in the cells from SCI mice except action potential decay slope (Fig. 3). We previously reported that the measured membrane properties of tonic and initial burst cells were indistinguishable in controls (Dougherty et al. 2005). When grouping these neuron populations, membrane potential, steady-state outward current, spike height, and action potential decay slope were all altered in cells from SCI mice. Unlike controls, the membrane properties of tonic and initial burst firing types differed within the cell population from SCI mice. Rheobase was lower (49 vs. 74 pA; \( P < 0.01 \)) and maximum firing frequency was higher (54 vs. 32 Hz; \( P < 0.0001 \)) in cells firing tonically. Moreover, the tonically firing
Evidence of spontaneous plateau potentials was seen in few cells from control mice (7%). However, 27% of cells from SCI mice displayed at least one spontaneous plateau potential during continuous recordings. Plateaus were most commonly seen in tonically firing cells. Their duration ranged from short (~50 ms) to very long duration (~50 s) although most (~75%) were ≤1 s in duration. Plateaus spontaneously occurred within a membrane potential range of −120 to −40 mV with values ranging from −80 to −15 mV. In a recording from a particularly active cell from a spinal transected mouse (Fig. 6), the heterogeneity of plateau duration and magnitude is obvious. The increased incidence of spontaneous plateaus suggests there may be an increase in the expression of PICs, which would also contribute to the higher firing frequencies seen with f-I curves (Kuo et al. 2006).

**Persistent inward currents in control cells**

PICs were examined using triangular voltage ramps (−110 to −10 mV) over 5 or 10 s. PICs were not evident in most neurons in control mice (−11 ± 4 pA; Fig. 7A). However, following blockade of voltage-gated K⁺ channels with intracellular Cs and extracellular 4-AP and TEA, PICs were seen in all cells tested (n = 27, Fig. 7B). For the rising phase, PIC amplitudes were −38 ± 5 pA in 5-s ramps and approximately double in 10-s ramps (−75 ± 20 pA), and the corresponding falling phase values were −48 ± 6 and −104 ± 22 pA in 5- and 10-s ramps, respectively. The membrane potentials at the peak amplitudes during rise (−40 ± 2 mV) and falling phases (−46 ± 3 mV) were comparable.

TTX blocked the majority of the PIC with peak current of the rising and falling phases reduced to 12 and 38% of the control (Fig. 7D). In comparison, riluzole, the persistent sodium channel blocker (Fig. 7E) reduced the peak amplitude of the rising phase to 60% of control. The descending phase was also reduced, to 70% of the peak amplitude of control (Fig. 7C).

The Ca²⁺ channel blocker Cd²⁺ blocked most of the rising phase of the ramp current (96%) and partially blocked the falling phase (left 39% of the control remaining; n = 2). Ni²⁺, a T-type Ca²⁺ channel blocker (n = 2), had no effect on either rising or falling phase PIC amplitude. The L-type Ca²⁺ channel blockers, nifedipine and verapamil, reduced both rising and falling phases of the voltage ramps. Nifedipine (n = 7) reduced the PIC to 49% of control values on rising and 68% on the falling phase (Fig. 7). Verapamil had weaker actions. In two of three cells tested, verapamil reduced PIC amplitude to 87 and 77% of control rising and falling phase values, respectively. In summary, both voltage-gated Na⁺ and Ca²⁺ currents contribute to PICs.

While pharmacological characterization of the conductances underlying PICs in neurons in SCI mice was not performed, under normal recording conditions, the responses to voltage ramps were not significantly different from controls (n = 32; Fig. 8A). However, in 28% of these cells, a high-threshold inward current is activated and can be seen in the falling phases of PICs (Kuo et al. 2006).
FIG. 3. Comparison of passive and active membrane properties in cells from control and SCI mice divided into 2 separable groups based on firing properties. ■ control means; ○ SCI means. Data are presented as means ± SE. *, statistical significance ($P < 0.05$). A: following spinal transection, tonic/initial burst cells had more depolarized membrane potentials, larger steady-state outward currents, and larger spike heights. B: the other passive properties measured (resistance, $\tau_m$, and cell capacitance) remained unchanged. C: voltage threshold, rheobase, peak inward current, and overshoot were statistically unchanged. While the rise slope was unchanged, the decay slope was less steep in cells from spinal transects.
(repolarizing) phase of the voltage-clamp ramp (Fig. 8A2). In these cells, the falling phase of the ramps had larger peak amplitudes than controls (−30 vs. −11 pA: P < 0.05). In long voltage step protocols (5 s), a high-threshold current with delayed activation and no inactivation was seen (Fig. 8B). These currents activated at approximately −30 mV, and maximum current varied but was usually 100–250 pA. None of these cells showed either accelerated firing during current steps, a sustained depolarization beyond current steps (Fig. 8C), or enhanced firing on the repolarizing side of a current ramp (Fig. 8D).

**DISCUSSION**

We sought to determine the effects of SCI on lamina I GABAergic neurons using a complete transection model of SCI. A subset of GABAergic neurons became more excitable following injury as seen by depolarized membrane potentials, larger spike heights, increases in firing frequencies, and increased incidence of spontaneous plateau potentials. In looking for the ionic basis for the plateau potentials, we found that all lamina I GABAergic neurons have PICs mediated by persistent Na⁺ and/or L-type Ca²⁺ channels. However, these channels are likely on distal dendrites because they emerged only after facilitating space clamp voltage control with the addition of K⁺ channel blockers. Notably, following spinal cord transection, a high-threshold, non-inactivating PIC emerges in approximately the same incidence of neurons at which spontaneous plateaus were encountered.

**Effects of injury on the spinal GABAergic system**

Approximately 2/3 of SCI patients suffer from chronic pain (Finnerup and Jensen 2004), and numerous studies have demonstrated a hyper-responsive sensory apparatus after SCI (Bennett et al. 1999; Drew et al. 2001; Hains et al. 2003b; Hao et al. 2004; Zhang et al. 2005). GABAergic control of spinal excitability becomes critical after the loss of descending inhibitory controls (Millan 2002), and dysfunction in spinal inhibitory systems has been implicated in altered nociceptive activity (Baba et al. 2003; Coull et al. 2003; Drew et al. 2004; Hao et al. 1992; Moore et al. 2002; Stiller et al. 1996). Caudal to chronic cord transection, GAD-67 expression increases (Tillakaratne et al. 2000) as does afferent-evoked GABAergic synaptic actions (Garraway and Mendell 2007). These processes are likely highly dynamic as increased GAD67 expression can be normalized by exercise training (Tillakaratne et al. 2002). Here, in lamina I, there was no difference in the number, apparent distribution, or incidence of functional subpopulations of EGFP⁺ neurons 8 days postspinalization.

**Excitability increases in a subset of GABAergic neurons following spinal transection**

Interestingly, changes in cellular properties following spinalization were largely restricted to a cell subpopulation of lamina I GABAergic interneurons, those that respond to depolarizing currents steps with tonic and initial burst firing. The observed changes all appear to function to increase cell excitability. In comparison, no changes were seen in membrane properties of lamina II GABAergic neurons following chronic constriction nerve injury (Schoffnegger et al. 2006). One of the more striking observations was a depolarizing shift in the membrane potentials of cells from SCI mice. It is possible that an injury-related change in the Cl⁻ gradient (Coull et al. 2003) may contribute to this. Because voltage threshold and rheobase were unchanged, synaptic and excitatory neuromodulatory events would be much more likely to recruit these neurons. Additionally, observed increases in spike height and outward current could contribute to an increase in excitability. An increase in spike height may be due to changes in voltage-gated Na⁺ or K⁺ channels (Chen et al. 1996; Kang et al. 2000; Melnick et al. 2004). The increase in spike heights is likely due to a decrease or
change in K⁺ channel properties. Because the rising slope of
the spike and peak inward currents remained unchanged
while action potential decay was faster, K⁺ channels re-
sponsible for fast repolarization (A-current) are implicated
(Chen et al. 1996; Kang et al. 2000). Alternatively, this
could be due to changes in the kinetics of Na⁺ channels or
a shift in the voltage dependence due to changes in auxiliary
subunits (McCormick et al. 1999; Meadows et al. 2002).
The increase in peak steady-state outward currents follow-
ing spinal transection suggests that one of the delayed
rectifier K⁺ channels is facilitated and is consistent with
faster firing frequencies (Melnick et al. 2004). Also even
though the changes were not significant, voltage thresholds
of cells from SCI mice tended to be more hyperpolarized.

Spinal cord transection causes firing frequencies of tonic
cells to increase

This is the first study to examine the effects of cord tran-
section on lamina I GABAergic neurons, and we show evi-
dence of an increased firing frequency in a subset of tonic cells. Increased spinal excitability and increases in cell firing rates
have been reported following cord injury (Hains et al. 2003a;
Millan 2002) and in pain models (Finnerup and Jensen 2004;
Pitcher and Henry 2000), and a loss of inhibitory function has been implicated in pain following injury (Burchiel and Hsu 2001; Wiesenfeld-Hallin et al. 1997) and pain in general (Malan et al. 2002; Millan 1999; Zeilhofer 2005). While there have been no studies directly examining changes in the excitability of inhibitory neurons, based on the aforementioned studies, one would predict reduced excitability. On the other hand, following the loss of descending inhibition, compensatory homeostatic mechanisms intrinsic to the spinal cord may warrant the increases as seen here, in GAD-67 expression (Tillakaratne et al. 2000) and in afferent-evoked GABAergic synaptic actions (Garraway and Mendell 2007). 

Increases in firing frequency were specific to tonic firing cells, presumably to result in stronger inhibitory actions on spinal circuits. Because only 11% of lamina I cells are local interneurons (Bice and Beal 1997), and GABAergic neurons comprise 1/4 of the lamina I population (Polgar et al. 2003; Todd and Spike 1993), some GABAergic cells must be propriospinal neurons. Most tonic and initial burst cells are the larger fusiform cells rather than small multipolar, single spike cells (Dougherty et al. 2005). Therefore tonic cells may be propriospinal neurons with more widespread projections. Hence their lesion-induced excitability increase may exert more widespread compensatory inhibitory actions. In contrast, single spike cells may be local circuit interneurons and less relevant to the control of and compensatory response to overall spinal hyperexcitability.

**Spontaneous plateaus are more frequent and PICs are evident in cells from SCI mice**

As seen in unidentified neurons in the substantia gelatinosa (Yoshimura and Jessell 1989) and deep dorsal horn (Derjean et al. 2003; Monteiro et al. 2006), only a small proportion of lamina I GABAergic neurons have spontaneous plateau potentials. However, a greater number of cells displayed plateaus following chronic cord injury. Plateau potentials depend on neuromodulation in motoneurons (Hounsgaard and Kiehn 1985). Interestingly, plateau potentials disappear after acute cord transection but re-emerge much more prominently in chronically spinal transected rodents (Li and Bennett 2003) and appear to be one mechanism that results in hyperreflexia and spasticity (Bennett et al. 2004).

PICs underlie the plateaus in motor (Carlin et al. 2000; Heckman et al. 2003; Hounsgaard and Kiehn 1989; Li and Bennett 2003), deep dorsal horn (Morisset and Nagy 1999), and ventral horn neurons (Theiss et al. 2007). In particular, L-type Ca$^{2+}$ currents and persistent Na$^{+}$ currents are responsible, both in normal (Hounsgaard and Kiehn 1989; Kuo et al. 2005) and in chronically transected (Li and Bennett 2003) animals. Both persistent Na$^{+}$ and persistent Ca$^{2+}$ currents have been demonstrated in lamina I tonically firing cells where they amplify and prolong depolarization (Prescott and De Koninck 2005).

PICs were not found in normal recording conditions in most cells from control or SCI mice. Following K$^{+}$ current blockade with Cs$^{+}$ (and in some cases 4-AP and TEA), persistent currents were seen in all cells tested. Although firing properties could not be measured in these cells, it is likely that PICs are present in all GABAergic cells in lamina I, regardless of firing type. This conflicts with reports that only tonic cells in lamina I have PICs (Prescott and De Koninck 2005). It is possible that differences in the protocols used to evoke the PICs, differences in the recording conditions, or bias toward excitatory cell types may be responsible for the conflicting results. Because the currents were evident only after K$^{+}$ channel blockade, it is likely that the channels underlying these currents are on the distal dendrites (Carlin et al. 2000; Hounsgaard and Kiehn 1993; Russo and Hounsgaard 1996). Both persistent Na$^{+}$ channel blockers and L-type Ca$^{2+}$ channel blockers were effective at reducing the currents. A model of PICs in tonic firing cells is shown in Figure 6.
firing lamina I cells predicts the activation of the Ca²⁺ current to be dependent on the persistent Na⁺ current and that the maintenance of the Na⁺ current will depend on the Ca²⁺ current activation (Prescott and De Koninck 2005). Here both TTX and Cd²⁺ individually are capable of eliminating most of the PICs, suggesting that these currents are largely interdependent to generate PICs.

After spinal transection, a high-threshold current with a delayed activation emerges in a subset of cells. It is possible that this current is required for the spontaneous plateaus seen since they are expressed in the same percentage of cells (28 and 27%, respectively). This current is similar to dendritic L-type calcium currents reported in other regions (Carlin et al. 2000; Mermelstein et al. 2000) and has threshold properties consistent with L-type Ca²⁺ channels (Huang 1989). Self-sustained depolarizations at the offset of current steps and increased firing frequencies during the hyperpolarizing phase of ramps are properties characteristic of cells which generate plateaus. Despite the increased incidence of plateaus and PICs in cells from spinal cord transects, none of these cells displayed self-sustained depolarizations or increased firing frequencies. It is possible that the PICs would become larger at longer durations after spinal cord transection (Li and Bennett 2003). Because the activation of these currents is slow, it is possible that their activation is too delayed to affect spiking during a current ramp. Additionally, the long somatic current steps may not have been depolarizing enough to activate these currents out in the dendrites, possibly explaining the lack of self-sustained depolarization.

**FIG. 7.** Persistent inward currents (PICs) in control cells emerge following K⁺ channel blockade and are partially blocked by both Na⁺ channel and Ca²⁺ channel blockers. A1: example of a response to a voltage-clamp ramp in normal recording conditions. A2: resulting currents after leak subtraction of A1. Stimulus voltage ramp is shown below. B: example of a response to a voltage-clamp ramp in the presence of K⁺ channel blockers. Raw trace is shown in B1 and leak subtracted in B2. C: summary of the effects of channel blockers on the amplitude of PICs. Darker bars represent the peak current of the depolarizing phase of the ramp and light gray bars correspond to the peaks in the hyperpolarizing phase of the ramp. Graphs and error bars are means ± SE. D: current resulting from voltage ramp (black) that was partially blocked by TTX (gray). E: example of control currents (black) partially reduced by riluzole (gray). F: L-type Ca²⁺ channel blockers, such as nifedipine, also reduce the persistent inward current.
Conclusion

In a subset of lamina I GABAergic neurons, spinal transection induces a more depolarized resting potential and increased firing frequencies and plateau potentials, supporting a resultant increase in neuronal excitability. The mechanisms responsible for altering excitability remain unknown. Because descending modulatory actions are predominantly inhibitory to dorsal horn (Millan 2002) and their actions are lost after chronic cord transection (see Hadjiconstantinou et al. 1984), an increased GABAergic neuronal excitability may result as part of a compensatory homeostatic response, albeit insufficient.

ACKNOWLEDGMENTS

We thank M. Sawchuk for expert technical assistance. Present address for K. J. Dougherty: Dept. of Neuroscience, Karolinska Institute, 17177 Stockholm, Sweden.

FIG. 8. A high-threshold current with slow activation is seen in some cells from SCI mice. A: examples of the responses of 2 cells from SCI mice to a voltage ramp. Both traces have been leak subtracted. Note the lack of PIC in the hyperpolarizing phase in A1 and the presence of a late activating PIC during the hyperpolarizing phase in A2. B: current response to 10-mV voltages steps from a holding potential of -80 mV. The slow activating current is seen at high thresholds. C: cells from SCI mice did not display self-sustained depolarizations in response to 5-s-long current steps. The cells were brought to approximately -80 mV prior to a 50-pA step. Cells either returned to baseline (C1) or hyperpolarized (C2) at the end of a step. D: response of a cell from a SCI mouse to a current-clamp ramp. Gray dashed line indicates the voltage threshold, which was -43 mV in this cell.
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