Riluzole decreases flexion withdrawal reflex but not voluntary ankle torque in human chronic spinal cord injury

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1Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago; 2Department of Physical Medicine and Rehabilitation, Feinberg School of Medicine, Northwestern University, Chicago; 3Department of Physical Therapy, University of Illinois at Chicago, Chicago; 4Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago; 5Department of Biomedical Engineering, Northwestern University, Evanston, Illinois; and 6Department of Biomedical Engineering, Marquette University, Milwaukee, Wisconsin

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Theiss RD, Hornby TG, Rymer WZ, Schmit BD. Riluzole decreases flexion withdrawal reflex but not voluntary ankle torque in human chronic spinal cord injury. J Neurophysiol 105: 2781–2790, 2011. First published March 23, 2011; doi:10.1152/jn.00570.2010.—The objectives of this study were to probe the contribution of spinal neuron persistent sodium conductances to reflex hyperexcitability in human chronic spinal cord injury. The intrinsic excitability of spinal neurons provides a novel target for medical intervention. Studies in animal models have shown that persistent inward currents, such as persistent sodium currents, profoundly influence neuronal excitability, and recovery of persistent inward currents in spinal neurons of animals with spinal cord injury routinely coincides with the appearance of spastic reflexes. Pharmacologically, this neuronal excitability can be decreased by agents that reduce persistent inward currents, such as the selective persistent sodium current inhibitor riluzole. We were able to recruit seven subjects with chronic incomplete spinal cord injury who were not concurrently taking antispasticity medications into the study. Reflex responses (flexion withdrawal and H-reflexes) and volitional strength (isometric maximum voluntary contractions) were tested at the ankle before and after placebo-controlled, double-blinded oral administration of riluzole (50 mg). Riluzole significantly decreased the peak ankle dorsiflexion torque component of the flexion withdrawal reflex. Peak maximum voluntary torque in both dorsiflexion and plantarflexion directions was not significantly changed. Average dorsiflexion torque sustained during the 5-s isometric maximum voluntary contraction, however, increased significantly. There was no effect, however, on the monosynaptic plantar and dorsiflexor H-reflex responses. Overall, these results demonstrate a contribution of persistent sodium conductances to polysynaptic reflex excitability in human chronic spinal cord injury without a significant role in maximum strength production. These results suggest that intrinsic spinal cellular excitability could be a target for managing chronic spinal cord injury hyperreflexia impairments without causing a significant loss in volitional strength.

The link to the full text of the article is available at the J Neurophysiol website.
teaus and currents blocks spastic reflexes (Li and Bennett 2003; Li et al. 2004).

Recent studies in humans have shown evidence that, like in animal models, PICs may also contribute to prolonged and amplified reflex responses in chronic SCI. For example, tests of lower limb flexion withdrawal reflex “windup” (i.e., progressively increasing responses to repeated, identical amplitude stimuli) produce long-lasting, hyperexcitatory reflex responses that may reflect the presence of plateau potentials in motoneurons, interneurons, or both (Hornby et al. 2003). Schmit and Benz (2002) demonstrated that a brief stretch of hip flexors in subjects with chronic SCI produced long-lasting, heteronymous, multijoint extensor spasms that likely activated an abnormally excitatory interneuronal pathway. Paired motor unit studies have also suggested that intrinsic motoneuronal PICs likely drive involuntary spasms (Gorassini et al. 2004). In addition, Norton et al. (2008) demonstrated that a brief cutaneous muscular stimulus produced a short-lasting motor unit firing response in spinally intact control subjects but a much longer lasting response in subjects with chronic SCI, and they attributed this response prolongation to motoneuronal PICs. Together, the results from these human studies reflect what is known about PICs from animal studies: PICs underlie plateau potentials, repetitive and self-sustained firing, and amplification and prolongation of synaptic input.

The objectives of this study were 1) to pharmacologically probe the contribution of intrinsic neuronal conductances to reflex excitability and voluntary motor control by reducing NaP currents with riluzole and 2) to determine the spinal locus of hyperexcitability (i.e., interneurons, motoneurons, or both). In particular, we chose to assess the impact of NaP currents on the polysynaptic flexion withdrawal reflex response, the monosynaptic electrically elicited H-reflexes, and voluntarily produced maximal contractions in the lower limb (about the ankle) in subjects with chronic incomplete SCI. Riluzole was recently shown to decrease spastic flexion reflexes in response to noxious and nonnoxious cutaneous stimuli in spinal cord-injured rats (Kitzman 2009). Our results support this finding and suggest that NaP does indeed have a strong effect on the amplitude of polysynaptic reflex responses, especially at the interneuronal level, and that the intrinsic excitability of spinal neurons could provide a novel and effective pharmacological target for the medical management of hyperreflexia and motor impairments in human chronic SCI.

Portions of this work have been presented previously in abstract form (Theiss et al. 2008).

MATERIALS AND METHODS

General experimental procedures. All studies and procedures were conducted in accordance with the Declaration of Helsinki and with the full approval of the Northwestern University Office for the Protection of Research Subjects Institutional Review Board in compliance with their guidelines for research involving human subjects. Informed consent was obtained in writing from all subjects before enrollment and participation in the research study. All studies were conducted in research laboratories at the Rehabilitation Institute of Chicago (RIC).

Briefly, the study design involved a double-blinded, single-dose, placebo-controlled oral administration of 50 mg of riluzole (Rilutek; Sanofi-Aventis, Bridgewater, NJ) given after the completion of the pretest experimental protocol. After an ~90-min wait (the approximate time to peak plasma concentration), a posttest repeating the same experimental protocol as the pretest was conducted. Both the pretest and posttest sessions were completed on the same experiment day. At least 7 days for a “washout” period were provided between experiment days. The riluzole and the placebo administration order was randomized, blinded by enclosure in identical-looking capsules, and packaged into experimental day A and experimental day B prescription bottles with sequential numbering by the RIC Pharmacy. Neither the subject nor the study staff knew which drug was being administered on which day, although the blinding code could be broken by pharmacy staff in case of a medical emergency.

Subject population. A total of seven subjects with chronic (>1 yr) motor-incomplete SCI participated in both drug and placebo testing days of this study. As shown in Table 1, two of the seven subjects were classified as AIS C, four were classified as AIS D, and one was assessed as between AIS C and D. Six of the subjects had not been taking antispasticity medications for more than 1 yr before enrollment in the study, and one of the subjects had not taken antispasticity medication for more than 14 days before participating in the first experiment day. Clinical evaluation of strength and spasticity were performed before subjects were transferred into the experimental apparatus, ~30–45 min before quantitative testing began. These clinical measures were assessed using the American Spinal Injury Association Impairment Scale (AIS) classification (Marino et al. 2003), Modified Ashworth score (knee extendors/knee flexors, 0–4 scale: with 0 being no impairment and 4 being severe spasticity), and the Spinal Cord Assessment Tool for Spasticity (SCATS; extension/flexion/clonus, 0–3 scale, with 0 being no impairment and 3 being severe spasms lasting longer than 10 s) (Benz et al. 2005). A summary of the subjects’ clinical features is presented in Table 1. Specific inclusion/exclusion criteria for participation included a nonprogressive lesion between C1 and T10, with below T10 excluded due to...
potential peripheral nerve damage/cauda equina injury. Subjects were medically stable, with no concurrent medical illnesses, were not concurrently taking medications for spasticity or pain, and had medical clearance from their primary internists or physiatrists to participate. Subjects were also excluded for significant cardiorespiratory, metabolic, orthopedic, or other neurological disease. Women of childbearing potential were not excluded, although women who were pregnant or nursing were excluded due to unknown or potential risks from the pharmacological agent to the fetus or nursing child. As determined by the subject’s primary physician or physiatrist, subjects were also excluded if there were any potential interactions of riluzole with other concurrent medications or if the subjects had a history of sensitivity to the test agents or their components.

Testing apparatus and evaluation of spastic reflexes and motor impairments. Measurements of electromyogram (EMG) activity and joint torques were used to quantify stimuli-evoked reflex responses and volitional motor strength. As previously described (Hornby et al. 2003, 2006) and shown in Fig. 1, subjects were seated in an adjustable chair with their test foot securely strapped to an instrumented footplate mounted on a 6-degree of freedom load cell (ATI, Apex, NC) attached to a mechanical fixture (Biodex Rehabilitation System 3; Biodex Medical Systems, Shirley, NY). Surface EMG electrodes (Delsys, Boston, MA) were attached to the skin above the muscle to measure the activity in the tibialis anterior (TA), soleus (SOL), medial gastrocnemius (MG), vastus lateralis or medialis, rectus femoris, and medial or lateral semimembranosus/ semitendinous muscles. EMG signals were amplified (×1,000) and filtered (20–250 Hz) online before acquisition. End-point torque signals were low-pass filtered (200 Hz). Customized LabVIEW software (National Instruments, Austin, TX) was used for data acquisition (sampled at 1,000 Hz) and control of electrical stimuli. Lower limb segment lengths and endpoint torques were used to calculate ankle, knee, and hip joint torques (Schmit et al. 2000).

Quantitative assessment of reflex excitability and voluntary motor strength. Torque and EMG measurements of the flexion withdrawal reflex response were used to assess reflex excitability. Flexion withdrawal reflexes were elicited by a controlled electrical stimulus train (biphasic, train of 10 1-ms pulses, 200 Hz, for 50 ms) delivered through a pair of bipolar electrodes placed 0.5–1 cm apart on the medial plantar arch of the foot. Flexion reflex “threshold” was determined by the smallest amplitude stimulus to produce a TA EMG response and at least 1 Nm of dorsiflexion torque in two of three stimulus trains given 20 s apart. The threshold stimulus intensity was determined at the beginning of each testing session. Flexion reflex responses to three stimulus trains at 20-s intervals were tested at 1, 2, 3, and 4 times the threshold to assess the response (EMG or torque) vs. stimulus intensity (stimulus-response) relation. Generally, most subjects perceived this stimulus as noxious.

H-reflex and M-wave EMG responses were elicited via a bipolar stimulating electrode with two 1-cm-diameter contacts located 3 cm apart from their centers to assess monosynaptic reflex responses and for normalizing TA and SOL EMG responses. Responses for plantarflexion were elicited by stimulation of the Tibial nerve at the popliteal fossa, and responses for dorsiflexion were elicited by stimulation of the common peroneal nerve branch just lateral to the head of the fibula. H-reflex and M-wave responses were measured by determining the H-reflex threshold and increasing the stimulus intensity in 1- to 2-mA increments until the M-wave amplitude no longer increased. (For additional certainty, the stimulus intensity was increased by 20% after this point for a supramaximal measurement.) Stimuli were delivered every 5 s. The maximal H-reflex and M-wave responses allowed for comparison of H-reflex maximum amplitude and M-wave maximum amplitude (Hmax/Mmax) ratios across subjects during different recording conditions. TA and SOL EMG responses were normalized to the amplitude of the maximal M-waves (Mmax).

Torque and EMG responses to maximum voluntary contractions (MVC) were also measured for assessment of subject ankle strength. With the knee extended, measurements of the volitional strength of the plantar flexors were made from an ankle position of 0° (neutral), and measurements of the volitional strength of the dorsiflexors were measured from an ankle position of 30° plantarflexion. Three trials each of maximal voluntary dorsiflexion and three trials of maximal voluntary plantarflexion were performed upon verbal cue. Subjects were verbally instructed and motivated to maintain these maximum contractions for ~5 s. Visual feedback of torque production was not provided, although subjects were generally consistent in their MVC torques. Peak MVC torque responses, duration about the peak, and average sustained torque produced during the MVC were measured and averaged over the three trials. To account for any differences in MVC duration due to instruction or reaction time variations from subject to subject, measurements involving torque over time were normalized to instruction duration and reported in relation to time in seconds.

Pharmacological administration. To control for diurnal fluctuations in reflex behavior and for subjective effects of agent administration, these studies were performed using a double-blinded, placebo-controlled, randomized design. Either a placebo or 50 mg of riluzole (Rilutek) were administered following the pretest quantitative evaluation. After a wait period of ~90 min, the posttest was performed. (Note: posttest clinical evaluations were performed before the subject was transferred into the experimental setup, ~45–60 min after riluzole administration. Since these clinical tests were performed ~30–45 min before the time to peak plasma concentration of riluzole, it is unknown whether an effective concentration of the drug had been reached by the time of these evaluations.) For each subject, pretests were performed at approximately the same time in the morning, and posttests were started at approximately the same time in the afternoon. During the posttest, the subjects repeated the entire protocol using a similar experimental paradigm and setup (subject positioning, electrode placement) as in the pretest. A drug washout period of at least 7 days was taken between testing days.

Data analysis. Data were analyzed off-line using custom-written analysis scripts in MATLAB (The MathWorks, Natick, MA). As stated above, all data were collected at a sampling frequency of 1,000 Hz. Torque measurements were smoothed by a 10-point moving window average, utilizing the MATLAB “filtfilt” function (a zero-phase delay filter) as an all-zero, finite impulse response (FIR) filter resulting in an effective cutoff frequency of 25 Hz. EMG signals were rectified and low-pass filtered at 40 Hz (using a 2nd-order Butterworth filter).
filter and the fililt function in MATLAB) for peak amplitude and duration calculations. Peak, onset latency, and duration of the dorsiflexion torque responses to the flexion withdrawal stimuli were calculated from the filtered data, and the results from the three stimulus trains were averaged. Peak torque response was measured as the maximum torque for each stimulus train. Onset latency was measured from the beginning of the stimulus train to the first point on the torque response to cross a threshold of the mean plus three standard deviations ($+3\text{SD}$) of the baseline torque measurement from the first 50 ms of each trial. The duration was calculated as the time between the onset of the response and the end of the response, defined as the point where the torque dropped below the threshold of mean $+3\text{SD}$ of the baseline. Peak, onset latency, and duration of the normalized and the low-pass filtered TA and SOL EMG responses were calculated in the same manner as the torque responses. The area of the normalized, filtered EMG responses was calculated using the MATLAB “trapz” function, estimating the integral of each EMG response from the calculated response onset to the calculated response end (through the response duration).

Strength measurements in both dorsiflexion and plantarflexion were assessed from the MVC protocol. Peak torque was measured as the maximum torque (in Nm) produced in the instructed direction, and average sustained torque was measured as the area under the torque curve during the instruction period, normalized to instruction duration, and reported in relation to time in seconds [in ($\text{Nm} \cdot \text{s}$/s)].

Statistical analysis. Results from the experimental protocol were compared between pretest and posttest, and results from drug administration are expressed in relation to placebo administration. (By performing pretests and posttests, subjects served as their own controls, and expression of double-blinded results in relation to placebo administration assessed the changes due specifically to riluzole administration.) Significance was determined using two-way repeated-measures ANOVA and Student’s $t$-tests. Levels of significance were set at $P < 0.05$ unless otherwise indicated.

RESULTS

The contribution of spinal neuronal NaP to hyperexcitable flexion withdrawal reflexes and voluntary strength was investigated in seven subjects with chronic incomplete SCI in a double-blind, placebo-controlled study design using a single-dose oral administration of 50 mg of riluzole (Rilutek) as a pharmacological probe. Our results demonstrate that NaP in chronic, incomplete SCI contributed to the stimulus threshold for the flexion-withdrawal response, the amplitude of the flexion withdrawal response, and the average sustained voluntary torque. NaP did not appear to have any effect on electrically elicited monosynaptic H-reflexes, however. These results suggest that the hyperexcitability in the dorsiflexion component of the polysynaptic flexion withdrawal response is related to NaP in spinal interneurons.

After riluzole administration, the threshold stimulus intensity for the flexion withdrawal response significantly increased compared with placebo administration [Fig. 2; $P = 0.03$, $n = 7$; riluzole: posttest minus pretest $= 2.0 \pm 2.0 \text{ mA (mean \pm SD)}$; placebo: posttest minus pretest $= 0.3 \pm 2.6 \text{ mA}$. Pretest threshold values for placebo and riluzole conditions were not significantly different ($P = 0.4$, paired Student’s $t$-test, $n = 7$).

Compared with placebo, riluzole administration produced a significant decrease in the peak amplitude of the ankle dorsiflexion torque component of the flexion withdrawal reflex, as shown in the example in Fig. 3. This decrease occurred over the series of stimulus intensities producing a significant difference in the stimulus-response relation with respect to placebo (posttest minus pretest difference, 2-way ANOVA with replication; $P = 0.0003$, $F = 14.9$, $n = 7$, Fig. 4A; posttest to pretest percent change, 2-way ANOVA with replication: $P = 0.002$, $F = 10.2$, $n = 7$, Fig. 4B). At each stimulus intensity, peak torque amplitude decreased $\sim 20\%$ (Fig. 4B). Torque response latency, duration, and area did not significantly change in posttest minus pretest values after riluzole administration with respect to placebo, although there was a significant percent change in torque response duration (2-way ANOVA with replication, percent change: $P = 0.03$, $F = 5.2$, $n = 7$). This change reflected an overall increase in response duration (averaged over all stimulus intensities) following placebo administration (posttest minus pretest $= 284 \pm 861 \text{ ms}$; percent change $= 28.7 \pm 62.2\%$, $n = 7$) and less of an increase in duration following riluzole administration (posttest minus pre-
the relationship for the placebo decreased only because the response to the 2× and 3× threshold changed very little (1% increase and 1% decrease, respectively). This stimulus threshold increase and slope decrease is consistent with in vitro reports that riluzole produces a rightward bias shift and gain decrease in the input-output relation in animal spinal neurons (Kuo et al. 2006; Theiss et al. 2007).

To assess the effects of riluzole on voluntary strength, we instructed subjects to produce MVCs in either dorsiflexion (DF) (at 30° of plantarflexion) or plantarflexion (PF) (at neutral ankle position) directions for ~5 s with verbal encouragement. The knee was fully extended in both situations. Overall, the subjects produced a pretest range of 2.6–21.6 Nm of peak DF torque (preplacebo: 14.5 ± 6.3 Nm; preriluzole: 13.4 ± 6.7 Nm) and a pretest range of 5.6–54.5 Nm of peak PF torque (preplacebo: 31.9 ± 15.6 Nm; preriluzole: 33.8 ± 16.4 Nm). Pretest peak DF and PF torque values were not significantly different between the placebo and riluzole conditions (pre-DF: P = 0.69; pre-PF: P = 0.13; paired Student’s t-test, n = 7). After riluzole administration, the pre- to posttest difference for DF peak torque was significant between the placebo and riluzole conditions (Fig. 6A), because after placebo administration, DF peak torque decreased significantly (P = 0.02, n = 7) from pre- to postadministration (preplacebo: 14.5 ± 6.3 Nm; postplacebo: 13.0 ± 6.0 Nm). After riluzole administration, however, DF peak torque did not significantly change (P = 0.79; preriluzole: 14.2 ± 6.4 Nm; postriluzole: 14.3 ± 7.0 Nm). The decrease in peak torque in the placebo condition, but

Fig. 4. Aggregate changes in flexion withdrawal reflex response to all stimulus intensities after placebo or riluzole administration. Zero on the y-axes is indicated by a dotted line. Values are means ± SD. A: riluzole administration decreased the peak flexion withdrawal reflex DF torque response at all stimulus intensities (1×–5× stimulus threshold). Significant differences between placebo and riluzole in the posttest minus pretest mean DF torque difference were seen at 3× and 4× threshold. *P < 0.05. B: riluzole administration decreased the peak flexion withdrawal reflex DF torque by ~20% at all stimulus intensities. Significant differences between placebo and riluzole mean DF torque %change were seen at 4× and 5× stimulus threshold. *P < 0.05. Note: the error bar for the placebo 1× stimulus threshold value has been truncated to show detail for the other stimulus intensities.

test = 13 ± 396 ms; percent change = 1.1 ± 31.7%, n = 7). TA EMG responses were consistent with the above results but were not significant.

After riluzole administration, threshold stimulus intensity increased and peak torque response decreased, changing the input-output (i.e., stimulus intensity-torque amplitude response) profile for the flexion withdrawal reflex (Fig. 5). After placebo administration (Fig. 5A), the pretest and posttest input-output relations almost overlapped. With riluzole administration (Fig. 5B), however, the posttest input-output relation shows a shift to the right and a decrease in the initial response slope (regression line fit to first 3 points) compared with the pretest relation. This initial slope decreased significantly following riluzole administration (29%, P = 0.015, n = 7, paired t-test) but did not decrease significantly following placebo administration (15%, P = 0.07, n = 7, paired t-test). As shown in Figs. 4B and 5A, the slope of the stimulus-response relationship for the placebo decreased only because the response to the 1× threshold stimulus intensity increased by 78% whereas
Riluzole administration did not significantly decrease mean peak torque during maximal voluntary contractions (MVCs). Values are means ± SD.

A: maximum voluntary DF mean peak torque production decreased significantly after placebo administration but did not significantly change after riluzole administration. *P < 0.05. B: maximum voluntary plantarflexion (PF) mean peak torque did not significantly change after placebo or riluzole administration.

Unexpectedly, although riluzole did not change the peak DF torque from pretest to posttest (note that peak DF torque decreased following placebo administration, however), the percent change in average sustained torque [normalized to command duration in (Nm·s)/s, see MATERIALS AND METHODS] increased significantly with riluzole administration compared with placebo (riluzole: 28 ± 34%; placebo: −12 ± 29%, P = 0.04). An exemplary illustration of this increase is shown in Fig. 7. Although peak DF torque did not significantly change after riluzole administration, the total torque produced during the MVC increased (Fig. 7A, top). For this subject, this change following riluzole administration may be related to the change in EMG activity (Fig. 7A, bottom), because the TA EMG is prolonged through the command (between the vertical dashed lines), whereas the cocontraction, represented by MG activity, was not present during the MVC in the posttest recording. Cocontraction between TA and MG (and sometimes SOL) during DF MVCs was observed in five of seven subjects, and MG activity in all five of these subjects was reduced following riluzole administration. Neither the average sustained PF torque nor other features of the PF MVC, however, significantly changed in either placebo or riluzole conditions (Fig. 7B).

The change in average sustained DF torque was also significantly related to initial voluntary DF strength. As shown in Fig. 8, the percent change in average sustained DF torque was negatively correlated to the pretest peak DF isometric MVC torque after riluzole administration (dashed line, $r^2 = 0.86$, repeated-measures ANOVA, $F = 30.4, P = 0.003$) but not after...
placebo administration (solid line, \( r^2 = 0.00006 \), repeated-measures ANOVA, \( P = 0.0003, P = 0.99 \)). This relationship indicates that the weaker subjects with the lowest initial voluntary DF isometric MVC torque exhibited the greatest increases in average sustained DF isometric MVC torque after riluzole administration, whereas the stronger subjects showed less of an increase in average sustained DF torque.

The percent decrease in flexion reflex peak DF torque after riluzole administration was also related to initial voluntary DF strength. As shown in Fig. 9, the percent change in peak flexion reflex DF torque was positively correlated to the pretest peak DF torque after riluzole administration (Fig. 9B) for \( 3 \times \) \( (r^2 = 0.84, P = 0.003) \), \( 4 \times \) \( (r^2 = 0.69, P = 0.02) \), and \( 5 \times \) threshold \( (r^2 = 0.74, P = 0.01) \). Riluzole had the greatest relative effect on the peak flexion reflex torque of the weaker subjects and less of a decrease for the stronger subjects. No relationship between change in reflex torque and initial strength was seen after placebo administration (Fig. 9A).

H-reflexes and M-waves were also tested in each subject for the TA and SOL. TA H-reflexes were able to be elicited in six of seven subjects, and SOL H-reflexes were present in all seven subjects. The peak-to-peak amplitude for \( H_{\text{max}} \) was compared with the peak-to-peak amplitude for \( M_{\text{max}} \) (ratio \( H_{\text{max}}/M_{\text{max}} \)). For both the TA and the SOL, \( H_{\text{max}}/M_{\text{max}} \) values were not significantly different pre- to posttest in either the placebo (TA: \( P = 0.83, n = 6 \); SOL: \( P = 0.86, n = 7 \)) or riluzole conditions (TA: \( P = 0.85, n = 6 \); SOL: \( P = 0.35, n = 7 \)).

Pretest to posttest changes in clinical measures of spasticity (Modified Ashworth score and SCATS) were minimal and not significantly different between placebo and riluzole administration. (Note: as mentioned in MATERIALS AND METHODS, clinical evaluations were performed before the subject was transferred to the experimental apparatus, 30–45 min before the time to peak plasma concentration of riluzole. Thus these changes are difficult to interpret.)

**DISCUSSION**

This study examined the contribution of intrinsic neuronal excitability, via NaP currents, to reflex activity, volitional strength, and motor coordination in chronic (>1 yr) incomplete SCI. The effects of riluzole administration on flexion withdrawal reflex responses and voluntary strength were assessed in seven subjects with chronic incomplete SCI using a double-blinded, placebo-controlled study design. The major results from this study demonstrated that, compared with placebo administration, riluzole significantly decreased the peak DF torque component of the flexion withdrawal reflex response and significantly increased average sustained isometric DF torque maintained during 5-s MVC trials that, in five of the seven subjects, was related to a decrease in antagonist cocontraction. In addition, changes in both flexion withdrawal response peak DF torque and MVC average sustained DF torque strongly correlated to initial isometric DF torque (as assessed by MVC peak torque). TA and SOL \( H_{\text{max}}/M_{\text{max}} \) ratios were not significantly different in either the placebo or riluzole conditions. As discussed below, these results suggest that NaP conductances at the interneuronal level greatly contribute to polysynaptic reflex excitability in chronic SCI.

**Contribution of NaP conductances to reflex excitability and motor control.** The first objective of this study was to pharmacologically probe the contribution of intrinsic neuronal conductances to reflex excitability and voluntary motor control by reducing NaP currents with riluzole administration. In cellular electrophysiology, riluzole is known to be a specific, progres-
sive inhibitor of the NaP current (Ptak et al. 2005; Urbani and Belluzzi 2000). This NaP current is essential for producing sustained, repetitive output (rhythmic firing) to sustained or slowly rising inputs (Kuo et al. 2006; Lee and Heckman 2001; Theiss et al. 2007). Decreasing NaP decreases repetitive-firing capabilities, reduces input-output gain, and increases input-initiated response threshold (Harvey et al. 2006b; Kuo et al. 2005, 2006; Ptak et al. 2005; Theiss et al. 2007; Urbani and Belluzzi 2000). The effects of riluzole administration on the flexion withdrawal response in our subject sample mimicked the increased threshold and gain reduction changes in the cellular input-output relation produced by reducing NaP. Because riluzole affects cellular excitability by inhibiting the sodium PIC, it is possible that riluzole inhibits NaP in human spinal neurons, as well, and that NaP may be a major contributor to hyperexcitable reflex responses and muscle activation discoordination in human chronic incomplete SCI.

In the drug-prescribing literature, riluzole is often listed also as an antiglutamatergic agent. However, a thorough review by Pittenger et al. (2008) revealed that many of the additional molecular effects attributed to riluzole have been obtained in vitro at concentrations that are much higher than would be therapeutically achievable. A riluzole dose of 50 mg bid produces a plasma concentration of ~0.9 –1.6 μM (Lacomblez et al. 1996), and the estimated average concentration in amyotrophic lateral sclerosis (ALS) patients receiving riluzole treatment is 1 μM (Urbani and Belluzzi 2000). Although we cannot completely rule out the possibility of antiglutamatergic activity, we would have expected to see decreases in every motor response that relies on glutamate for synaptic transmission (e.g., responses relayed by excitatory afferents including flexion withdrawal responses and H-reflexes, voluntary activation of motoneurons, etc.) if this was the primary mechanism of riluzole action.

Spinal locus of riluzole activity and reflex hyperexcitability. The second objective of this study was to determine the spinal locus of hyperexcitability (i.e., interneurons, motoneurons, or both). In animal studies, riluzole has been shown to reduce NaP and decrease cellular excitability in both spinal motoneurons (Kuo et al. 2006) and interneurons (Theiss et al. 2007). All of our experimental protocol (flexion withdrawal, MVCs, H-reflexes) tested motoneuron excitability, at least to some degree. Interneuronal excitability was primarily evaluated by testing the flexion withdrawal reflex, which involves polysynaptic interneuronal pathways. In addition, MVCs also allowed for examination of preserved corticospinal activity. The decrease in the polysynaptic flexion withdrawal reflex response without decreases in peak MVC torque or relative amplitude of H-reflexes strongly suggests that in this study of subjects with incomplete SCI, decreasing NaP with riluzole had the strongest effect on spinal interneurons. The finding that riluzole did not decrease the amplitude of peak MVC torque was unexpected, since NaP has a profound effect on motoneuron excitability in animal studies. An additional unexpected result was that average sustained DF MVC torque significantly increased after riluzole administration compared with placebo. In five of our seven subjects (see example in Fig. 7), this was accompanied by, and possibly attributable to, a decrease in antagonist cocontraction, a significant factor in motor discoordination that might have interneuron involvement. It is possible that the decrease in TA EMG accompanied by the increase in PF muscle EMG seen in these subjects was mediated by classic autogenic inhibition from Ib/Golgi tendon organ spinal input. The underlying function of this inhibition of homonymous muscle activity with facilitation of antagonist muscle activity involves group I nonreciprocal interneurons that receive predominant input from both group Ia and group Ib afferents (Jankowska 2001). In our study, all MVCs were isometric with a fixed ankle angle (thus Ia muscle spindle activation was minimal), and the foot was secured by dorsum straps to the footplate (thus providing additional tonic cutaneous input during DF MVCs). Cutaneous input has been shown to enhance the input from group Ib afferents (Powers and Binder 1985) to spinal neurons, so it is possible that in our experimental setup, Ib input to already highly excitable group I interneurons was additionally facilitated. A presumably disynaptic reciprocal facilitation of ankle PF muscles by ankle DF muscles has also been previously reported in subjects with spasticity after SCI and hemiplegia after stroke (Crone et al. 2003). The decrease in cocontraction following riluzole administration, then, may also be the result of the decrease in spinal interneuron excitability.

The stronger action of riluzole on polysynaptic reflex pathways but not on direct motoneuron excitability (e.g., peak MVC) may also point to a difference in the relative impact of NaP amplitude to interneuron vs. motoneurons excitability, or a difference in sensitivity to riluzole in these neurons. Previous work has shown that spinal neurons, interneurons especially, exhibit varying amounts of NaP that are directly correlated to their repetitive firing capabilities: cells with larger amplitude NaP have strong repetitive firing, whereas cells with little NaP only respond with a few action potentials regardless of the duration of the input (Theiss et al. 2007). It is possible that human spinal interneurons may be more reliant on NaP PICs for sustained output excitability and that decreasing NaP with riluzole will then have a more profound and noticeable effect in these cells. Interneurons may also simply be more sensitive to riluzole than motoneurons. For example, in respiratory pacemaking, some cells have been shown to be more sensitive to riluzole than others, suggesting a larger reliance on NaP for firing output, whereas others might be more reliant on CaP for their rhythmic activity (Pena et al. 2004).

Voluntary strength related effects of riluzole and implications for SCI. In this study, the relative effect of riluzole was strongly correlated to lower limb volitional strength. Lower limb strength may provide an inference to the extent of the spinal injury, although contributing factors such as muscle mass prior to injury, time since injury, and amount of atrophy may confound this assumption. Our subjects had intact peripheral nerves, so atrophy from denervation was likely minimal. Presumably, less paralysis would result in less atrophy. If anything, atrophy or greater percent decrease in muscle mass would only broaden the range of strength and not detract from this relationship. In subjects with less preserved volitional strength (lower peak MVC DF torque), a greater decrease in the DF component of the flexion withdrawal response and a greater increase in the average sustained MVC DF torque was a profound finding. This result may demonstrate a relationship between cellular hyperexcitability and the amount of preserved descending input, or the “completeness” of the injury. For example, in subjects with less preserved pathways (e.g., less voluntary strength), riluzole had a greater relative effect on the...
DF torque amplitude during the flexion withdrawal reflex response, whereas in subjects with more preserved pathways (e.g., greater voluntary strength), riluzole had a smaller relative effect. Further investigation is necessary to determine whether this effect would generalize to other joints. It is possible, as described above, that in more complete injuries, interneurons may rely more heavily on NaP for processing synaptic input and producing repetitive firing output. In addition, it is also possible that there is more NaP in cells with less preserved descending input as a result of an adaptation to the lack of this synaptic or neuromodulatory input. For example, recent studies have shown that PICs in chronic SCI models become supersensitive to serotonin (Harvey et al. 2006a; Li et al. 2007) and norepinephrine (Rank et al. 2007). In more complete injuries, it is possible that cells with an increase in the loss of descending input compensate more with a higher degree of supersensitivity adaptation. Riluzole, then, is not necessarily taking the place of descending input, but instead is providing a decrease in the adapted cellular excitability. Consistent with animal studies, decreasing NaP amplitude could decrease the amplification and prolongation of the synaptic input (Prescott and De Koninck 2005), increase neuronal input threshold for the onset of repetitive discharge, and decrease the discharge frequency for any given input, effectively decreasing input-output gain (Kuo et al. 2006; Theiss et al. 2007). Thus riluzole is not acting as a descending inhibitor or a neuromodulator but is acting on the end result of modulation or disinhibition (i.e., the activation and strength of intrinsic PICs) (Heckman et al. 2008).

The implications of the effect size relating to predrug strength are that pharmacological interventions targeting intrinsic excitability may have greater effectiveness to reduce hyperexcitability in weaker individuals. In addition, riluzole did not decrease DF or PF maximum voluntary strength measures in our study, suggesting a possible reduction in a potentially problematic reflex without the loss of strength often associated with other antispasticity medications. As for the potential for reducing spasticity in chronic SCI by decreasing intrinsic neuronal excitability, our results suggest that hyperexcitable, polysynaptic reflexes can be decreased by a drug that reduces intrinsic neuronal excitability through NaP. This opens up a novel target for the alleviation of spastic reflexes, separate from providing central inhibition (e.g., by baclofen) or inhibitory neuromodulation (e.g., by tizanidine).

Limitations. Limitations of this study included sample size, heterogeneity of subject sample, short-term vs. long-term effects of riluzole administration, and functional consequences. The primary limiting factor for the subject sample size in this study was the difficulty in recruiting subjects that were not restricted by the exclusionary criteria of concurrent use of antispasticity or pain medications. For this study, only subjects who were not taking antispasticity or other centrally acting medications (e.g., for the treatment of pain or depression) were recruited and qualified to participate. Despite the small number of subjects, however, the decrease in flexion withdrawal torque response was still significant. For individuals who experience larger and more troublesome spastic impairments that necessitate treatment by antispasticity and antipain medications, the effects of riluzole might be different, but we would expect that riluzole would significantly reduce hyperexcitable reflex responses in subjects with more severe spasticity, as well, perhaps even to a greater extent. Even though the sample size was limited, the effects of riluzole were uniform across subjects. For example, in the flexion withdrawal response, riluzole increased the stimulus threshold current 9–50% in six of seven subjects (in 1 subject, the threshold current decreased, although the decrease was less than with placebo, –12% with riluzole compared with –18% with placebo). In addition, riluzole consistently decreased peak DF torque responses at 2–5× threshold in six of seven subjects (in 1 subject whose stimulus threshold increased, riluzole decreased peak DF torque responses in only 1×, 4×, and 5× threshold). Placebo administration, conversely, produced mixed changes: increases in peak DF torque responses between 1 and 250% or decreases of <20%. Overall, however, riluzole’s effect compared with placebo was a greater increase in stimulus threshold and a greater decrease in peak DF torque response (with the exception of the single subject as noted).

Another possible limitation was that our subject sample was heterogeneous, displaying a range of AIS classifications, varying levels of lower limb clinical spasticity measures (e.g., Ashworth scores), and varying levels of voluntary ankle strength. Although this was not a homogenous group, this range did allow for comparison across the ranges of injury level, spasticity, and strength. Intersubject comparisons demonstrated significant correlations between initial voluntary DF strength (peak isometric DF MVC torque) and increases in average sustained voluntary DF torque and also between initial voluntary DF strength and decreases in flexion withdrawal DF torque responses following riluzole administration. In addition, the fact that the overall results were significant despite the heterogeneity is in itself remarkable and may speak to the robustness of the effect.

It is possible that long-term effects of chronic riluzole administration might yield different results than what we have reported above. Additional studies need to be done before this drug is utilized as part of a daily regimen to manage spasticity. Riluzole may be useful as an “as-needed” medication, but again, more studies need to be done to test its efficacy in this capacity.

Finally, although we have reported a decrease in the ankle component of a polysynaptic reflex response without a decrease in volitional ankle strength, the functional effects of riluzole administration need further study. Questions about possible detriments to locomotion (e.g., toe clearance) are still open to investigation.

Even with these remaining questions, this study provides insights into the mechanisms and spinal locus of hyperexcitable polysynaptic reflexes in chronic SCI. Significant results were obtained even with a small sample size from a heterogeneous population, and further investigation based on these findings is needed to shed light on the practical and appropriate use of drugs that target the intrinsic excitability of spinal interneurons to manage spasticity in chronic SCI.

Conclusions. This study is the first to show the effects of riluzole on hyperexcitable reflexes and strength in chronic human SCI. Overall, results from this study suggest that cellular excitability via persistent sodium conductances in spinal interneurons may contribute to hyperexcitable reflex responses and that cellular excitability is a potential target for the medical treatment of spasticity/hyperexcitable reflex responses. Riluzole may also present a tool to treat spasticity as an adjunct to physical therapeutic interventions. Using riluzole...
to control spasticity without a generalized reduction in strength may allow for additional benefit from locomotor training or other interventions to improve functionality. As previously suggested by Gracies et al. (1997), intrinsic cellular excitability presents a novel target for pharmacological intervention, and impairments such as hyperactive reflexes and motor discoordination might be reduced by targeting intrinsic neuronal excitability, facilitating the potential to improve function for individuals with chronic SCI.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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