Contribution of Muscle Afferents to Prolonged Flexion Withdrawal Reflexes in Human Spinal Cord Injury

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INTRODUCTION

Flexor spasms are prevalent in chronic human spinal cord injury (SCI) and can present a significant limitation to functional motor behaviors. After painful or innocuous skin stimuli, flexor spasms are characterized by long-lasting, coordinated muscle activity associated with flexor spasms involves self-reinforcing flexion reflex circuitry. Once a flexion reflex has been initiated, the resultant reflexive muscle contraction activates load-sensitive muscle receptors, which have an additional excitatory effect on flexion reflex pathways (Schmit et al. 2000). Effectively, a positive feedback system would be established in which transmission of the force-mediated afferent signals to hypereexcitable spinal circuitry could further excite flexion reflex pathways. This system resembles self-reinforcing reflex pathways that have been postulated to enhance extensor muscle activity during locomotor behaviors in felines and humans with and without SCI (for review, please see Dietz and Duysens 2000).

Evidence for the initiation of flexion reflexes by muscle afferent stimuli has been demonstrated in the acute, decerebrate cat after dorsal spinal hemisection. Non-noxious stimuli associated with isometric contraction (Cleland et al. 1982) or passive muscle stretch, particularly at longer muscle lengths (Cleland and Rymer 1990), have been shown to generate motor behaviors quantitatively similar to flexion reflexes. Such multi-joint reflexes after single joint perturbations are the basis for the well-characterized clasp-knife reflex (Burke et al. 1972; Rymer et al. 1979) and are thought to be elicited primarily by load- and stretch-sensitive, group III–IV, and nonspindle group II, muscular-free nerve endings (Cleland et al. 1990).

Similarly, passive stretch and loading of ankle plantar- or dorsiflexors has been shown to elicit flexion reflexes in chronic human SCI (Schmit et al. 2000). The observed multi-joint reflexes are nearly identical to those evoked by electrocutaneous stimuli (Schmit et al. 2000) and are not correlated to the velocity of ankle rotation (Schmit et al. 2002). The magnitude of the responses is correlated with the degree of passive ankle loading generated near the end range of movement, indicating a possible role for force-sensitive muscular afferents (group Ib, II–IV). Although force-sensitive afferents are a likely contributor to the flexion response, muscle-length-sensitive afferents, joint afferents, and cutaneous receptors are also likely to be activated by imposed movements and thus cannot be completely excluded.

In this study, we used intramuscular (IM) stimulation to investigate the role of load-sensitive muscle afferents in flexion withdrawal reflexes in human subjects with SCI. We postulated that flexion reflexes could be triggered by electrical activation of the muscle. The proposed mechanism for this reflex was the generation of muscle force, which would activate the load-sensitive muscle afferents, triggering a flexion reflex. The
latency of the reflex response was used to distinguish whether the response occurred via directafferent stimulation or indirectly by force generation in the muscle. We further hypothesized that the magnitude of the flexor spasms would be correlated with the muscle force generated during the stimulation. Evidence of a load-sensitive contribution to the initiation of flexion reflexes could indicate a possible role for force feedback in prolonging flexor spasms in chronic human SCI.

METHODS

Subjects

Thirteen subjects (2 females, 11 males) were recruited into this study through the outpatient clinics of the Rehabilitation Institute of Chicago. All subjects (age range: 19–62 yr) presented with traumatic or nontraumatic, nonprogressive SCI. Subjects were recruited from five centers across the country (Hornby et al. 2003; Schmit et al. 2002, 2003). Subjects were selected to participate only if volitional control was absent in their lower extremities (i.e., motor complete lesions with American Spinal Injury Association Classification A or B). Criteria for exclusion included: multiple CNS lesion sites, history of lower extremity peripheral nerve injury, the presence of skin breakdown, and/or concurrent illness limiting the capacity to conform to study requirements. Seven subjects were currently prescribed oral anti-spastic medications, including baclofen (range: 30–160 mg/d), benzodiazepine (5–20 mg/d), and tizanidine (4–12 mg/d). Consent was obtained for each subject. All procedures were conducted in accord with the Helsinki Declaration of 1975 and approved by Institutional Review Boards of Northwestern University and Marquette University.

Experimental design

Details of the experimental setup have been described previously (Hornby et al. 2003; Schmit et al. 2002, 2003). Subjects were transferred to an adjustable-height chair of the testing apparatus (Biodex Rehabilitation/Testing System 2; Biodex Medical Systems, Shirley, NY). The foot of the tested extremity was placed in a modified footplate attached to a 6 df load cell and secured using a heel strap and a clamp placed on the dorsum of the foot. The load cell was used to measure the isometric joint torques after cutaneous or IM electrical stimulation. Hip (range: 85–100°), knee (90–120°), and ankle (110–130°) angles were measured and unchanged throughout the experiment. The length of the thigh (greater trochanter to lateral epicondyle) and shank (lateral epicondyle to lateral malleolus) segments and the distance from the ankle joint to the load cell were measured. All force/torque signals were low-pass filtered (200 Hz) and sampled at 500 Hz. Isometric hip, knee, and ankle joint torques were calculated in the sagittal plane using equations described previously (Schmit et al. 2000).

Surface electromyograms (EMGs) were recorded from the tibialis anterior (TA), medial gastrocnemius (MG), rectus femoris (RF), and medial hamstrings (MH; semimembranosus/semitendinosus) in all subjects. Active bipolar electrodes (model DE2.1, Delsys, Boston MA) were applied to lightly abraded, degreased skin over the muscle belly near the approximate motor point. Signals were amplified (10,000 times), low-pass filtered (20–450 Hz), and sampled at 500 Hz using data-acquisition cards (National Instruments, Austin TX) on a personal computer.

Cutaneous and IM stimulation of the selected lower extremity muscles [TA or gastrocnemius (GS)] was performed during all experimental sessions. Cutaneous stimulation was performed via bipolar surface electrodes (Blue Sensor, Medicotest, Rolling Meadows, IL) placed ~1 cm apart at the medial arch or first web space and at selected sites along the Shank, which varied according to the experimental session (see details in the following text). Bipolar IM stimulation was performed after implantation of fine wires into the muscle belly of the selected muscle group(s). Sterilized, 0.0055-in diam, stainless steel wires (California Wire, Grover City, CA) were threaded through 23-gauge hypodermic needles and bent to >90° at the inserted end to reduce movement during contraction. Two centimeters of insulation was removed at both ends of the wire to reduce electrical impedance. The approximate location of the muscle motor point was determined by visualization of maximal twitch contractions after delivery of brief (1-ms duration) electrical stimuli applied to the skin overlying the muscle at one-third to one-half distance from muscle origin to insertion. For IM TA stimulation, each wire was inserted ~2 cm apart (i.e., electrodes inserted ~1 cm away from motor point), spanning the motor point perpendicular to muscle fiber arrangement. For IM stimulation of the GS, one fine wire electrode was inserted into the medial and lateral heads at the motor point and stimulation of the entire GS muscle was performed. Stimulation was triggered by a custom-made computer program and delivered through a constant current stimulator (Model DS-7A, Digitimer Stimulator, Hertfordshire, UK). The site and parameters of stimulation (i.e., amplitude, frequency) were varied according the experimental protocol detailed below.

Experimental protocol

To minimize habituation of flexion reflexes during a 2-h experimental session, three test paradigms were performed on subgroups of the subject population.

SESSION 1 PROCEDURES. In 13 subjects, cutaneous stimulation was performed at multiple sites across the lower extremity, and the resultant isometric reflex torques were compared with those obtained after IM TA stimulation. The electrical stimulus train for the first protocol consisted of a 200-ms, 50-Hz pulse train composed of 10 monophasic pulses (each pulse of 1-ms duration with 19-ms interpulse intervals). Stimulus-response curves were first generated by randomly varying the intensity of stimulation (0–20 mA at 5-mA intervals; 30–50 mA at 10-mA intervals) while recording EMGs and isometric joint torques. Stimulation trains at variable current amplitudes were repeated three times, with 20-s intervals between stimuli to minimize temporal summation or habituation (Hornby et al. 2003). Electrocutaneous stimulation was performed at three separate locations: at the medial arch or at the web space between the first and second digits (when arch stimulation did not elicit reflex activity), at the surface of the TA near the inserted fine wires, and at the surface of the Tibia directly medial to the surface TA stimulation site. Stimulation at different cutaneous sites elicited quantitatively similar flexion withdrawal patterns as measured by relative proportions of ankle and hip torques (cf. Schmit et al. 2003). Further, flexion reflex responses were compared with those elicited via IM stimulation by comparing the ratio of ankle to hip reflex torques (Schmit et al. 2003).

SESSION 2 PROCEDURES. Eleven of 13 subjects participated in the second experiment in which IM stimulation parameters were varied to modulate the force generated by the TA muscle (hereafter referred to as the “stimulus-induced muscle torque”). With a constant stimulus frequency (50 Hz), the stimulus amplitude was first varied from the minimal current necessary to produce muscle contraction (i.e., motor threshold) to the current at which the dorsiflexion torque reached a plateau (range: 0.5–50 mA across subjects). Subsequently, using a stimulus current at two to three times (mean = 2.68 times) flexion reflex threshold, the stimulus frequency was varied from 5 to 50 Hz to alter the stimulus-induced muscle torque, which included both unfused and fused tetanic contractions. Both the stimulus-induced muscle torque at the ankle and the ankle and hip flexor reflexes (“reflex torques”) were measured as described in the following text.

SESSION 3 PROCEDURES. In the third set of experiments (9/13 subjects), IM stimulation was performed in the GS using parameters.
identical to those used for the TA (session 1). Reflex responses were compared with those elicited after electrocutaneous stimuli applied to the arch/first web space stimulation and to the posterior surface of the calf.

Data collection and analysis

Despite reports of the presence of distinct short- and long-latency flexor reflexes in individuals with chronic, complete SCI (Roby-Brami and Bussel 1987), we were unable to identify two separate bursts of flexor muscle activity following cutaneous or IM electrical stimulation. The entire flexor reflex activity was therefore analyzed in all trials (Hornby et al. 2003; Schmit et al. 2003). Stimulus artifacts at the TA or GS prohibited quantification of EMGs during reflex behaviors. Analysis was therefore confined to hip and ankle joint torques generated during the flexion reflexes (knee flexion torques were inconsistent and often negligible) (Schmit et al. 2000). Hip and ankle torque signals were low-pass filtered at 25 Hz using a fourth-order Butterworth filter.

To quantify flexor reflex activity after cutaneous stimuli, the single, peak (maximum) ankle and hip torques were identified after the stimulus train. For IM stimuli, identification of flexor reflex torques was complicated at the ankle by the stimulus-induced muscle torque. To differentiate between reflex and stimulus-induced muscle torques generated during the flexion reflexes (knee flexion torques were inconsistent and often negligible) (Schmit et al. 2000). Hip and ankle torque signals were low-pass filtered at 25 Hz using a fourth-order Butterworth filter.

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RESULTS

Flexion reflexes after IM TA versus cutaneous stimulation

In the first experimental paradigm, stimulation at the medial arch or first web space of the foot elicited flexion reflexes in 13 subjects, observed as EMG activity of flexor muscles (specifically the TA) and flexion moments generated at the ankle and hip. In 11 of 13 subjects, stimulation of the TA through IM fine-wire electrodes generated an initial increase in ankle dorsiflexion torque as expected followed by a delayed, coordinated pattern of hip flexion and ankle dorsiflexion torques with variable EMG activity. In these 11 subjects, threshold currents for generation of flexion reflexes using cutaneous (arch) or IM stimulation were correlated significantly (R = 0.83; P < 0.01), although mean thresholds were not significantly different (Table 1). Despite differences in recording sites and stimulating electrodes, the significant correlation indicates that the generation of flexor reflex behaviors after IM or arch stimulation is at least partly dependent on the excitability of flexor reflex pathways in different subjects. Further, in the two subjects who did not generate flexor responses at the highest level of IM stimulus current, the amplitudes required to generate flexor reflexes using electrocutaneous stimuli were relatively high (>30 mA), indicating that the level of excitability of flexion reflex pathways may not have been sufficient to trigger reflexes with IM stimulation.

For electrocutaneous stimuli, the mean threshold for flexor reflex generation was 8.7 times the sensory perception threshold (1.5 ± 0.4 mA) as determined in five subjects without neurological injury. With electrical IM stimulation, the mean flexor reflex threshold was 4.12 times greater than motor threshold (SD = 3.17 times; range: 1.0–10 times). Notably, flexion reflexes were not elicited below motor threshold for any of the subjects tested using IM electrodes. Figure 1 demon-
strates an example of the EMG activity of TA, MG, RF, and MH muscles and ankle, knee, and hip torques in response to a 20 mA stimulus applied cutaneously to the first web space (Fig. 1A) and intramuscularly to the TA (Fig. 1B).

Although IM stimulation triggers a multijoint flexor reflex response, it is not clear whether the response is identical to the electrocutaneous reflex response. For example, because the trigger for the responses included skin and muscle afferents, different reflex pathways may be activated. In addition, the ankle musculature might be preferentially activated because of potentiation of active muscle fibers or manifestation of prolonged motoneuron activity generated by direct motor nerve stimulation or reflex activation (e.g., Collins et al. 2001, 2002). This phenomenon could result in a relative higher ankle torque for IM stimulation than cutaneous stimulation.

To assess whether direct stimulation of the TA contributed to the measured ankle torque responses during flexion reflexes, we compared the stimulus-response relation of peak ankle and hip torques between the different stimulation sites. Figure 2 shows the mean stimulus response relationships for both electrocutaneous foot and IM TA stimulation across a population of six subjects in which flexion reflex activity could be observed across a broad (10- to 50-mA current amplitude) range of stimuli (i.e., only data from subjects with both IM and cutaneous flexor reflex threshold ≤10 mA were utilized). Stimulus-response curves demonstrated a steeper rise in hip and ankle torques with increasing stimulus intensity applied cutaneously versus intramuscularly although responses at 10 mA were nearly equivalent. Specifically, across the range of stimulus amplitudes, the range of ankle to hip torque ratios was 1.38–2.00 after arch/first web space stimulation and 1.48–1.88 after IM stimulation with only small differences observed at any stimulus intensity (P > 0.30 at all stimulus amplitudes). This latter finding indicates that ankle dorsiflexion torque was not preferentially greater after IM TA stimulation, and focal TA activity after IM stimulation did not substantially alter the measured reflex response.

While flexion reflexes after IM stimulation were never observed below motor threshold (i.e., stimulus-induced muscle torque always preceded the reflex torque), the possibility of direct afferent or cutaneous stimulation causing flexion spasms cannot be discarded. To assess the possible contribution of cutaneous afferents to triggered spasms, we delivered electrocutaneous stimuli directly above the IM stimulation site or directly medial on the surface of the tibia. These responses

| Table 1. Threshold stimulation and hip torque spasm latencies after IM TA and electrocutaneous stimulation at multiple sites |
|---------------------------------|---------------|----------------|----------------|
| Stimulation Site                | Threshold Current | Hip Onset Latency | Hip Max Latency | Hip Onset Latency | Hip Max Latency |
| Intramuscular TA                | 12 ± 4.6        | 193 ± 60         | 313 ± 173       | 184 ± 100         | 337 ± 280       |
| Arch/1st web space              | 13 ± 7.6        | 132 ± 87*        | 188 ± 75*       | 145 ± 28          | 214 ± 27*       |
| Surface TA                      | 18 ± 8.8*       | 139 ± 56*        | 214 ± 53*       | 155 ± 29          | 222 ± 52*       |
| Surface tibia                   | 25 ± 12*        | 147 ± 29*        | 240 ± 36*       | 168 ± 32          | 250 ± 29        |

Flexion reflex thresholds and latencies of onset and peak hip torques at 50 mA and 2× threshold are shown. Repeated-measures ANOVA and post hoc Tukey-Kramer tests were performed to establish individual statistical differences at P < 0.05 (*) compared between intramuscular (IM) versus cutaneous stimulation at different sites only. Statistical differences were observed between threshold of flexion reflexes after IM stimulation and cutaneous stimulation at the tibialis anterior (TA) and tibial surface, but not at the medial arch/first web space. Significant differences in latency to onset and peak hip torques were detected at 2× threshold stimulus intensities but not at higher (50 mA) intensities. Values are averaged across all subjects with flexion withdrawal reflexes after IM stimuli (n = 11) and presented as means ± SD.
were compared with those generated after arch/first web space or IM TA stimulation. Figure 3 provides an example of flexor reflex behaviors in one subject after cutaneous stimulation at multiple sites along the lower extremity and after IM TA stimulation. To facilitate comparison of torque response, records of TA EMG and ankle and hip joint torque responses to medial arch stimulation are overlapped with those of the same subject after IM TA stimulation to facilitate comparison (Fig. 3A). Electrocutaneous stimulation overlying the tibia also generated a substantial flexor reflex (Fig. 3B) with minimal response generated after surface TA stimulation (Fig. 3C).

Across all subjects, two prominent differences in the flexion reflex behaviors with IM versus cutaneous stimulation were identified. First, the thresholds for flexion reflexes were greater after direct cutaneous stimulation at the TA or tibia as compared with medial arch or IM stimulation (Table 1). The threshold differences between cutaneous sites may be due to a reduced receptor density at the anterior shank versus the foot. These observations, coupled with the depth of the IM electrodes (described in METHODS), suggests that IM stimulation likely activates muscle afferents (directly and/or indirectly after muscle force production), rather than cutaneous receptors, to elicit flexion reflexes.

A second prominent difference between flexion reflexes after IM versus cutaneous stimulation was the latency of the response, reflected in differences in the onset and peak torques measured at the ankle and hip. As shown in Fig. 3A, the latencies of the peak ankle and hip torques after IM stimulation (black trace) were longer than those after cutaneous arch stimulation (gray trace). For IM stimulation, the flexion reflex (noted by simultaneous ankle and hip flexion torques) was not observed until after the decline of the stimulus-induced muscle torque. This latency difference was consistent between IM and all cutaneous stimuli, including the arch, TA, and tibial stimulus sites.

Mean values of the latency of hip torque onset and time to peak for reflex torques generated at both a relative (2 times flexor reflex threshold) and absolute (50 mA) stimulus amplitude are provided in Table 1. Only hip torque measurements are presented, as reflex onset was difficult to assess using TA EMG or ankle torque data during IM TA stimulus trials. The data demonstrate a significantly longer latency of flexor reflexes after 2 times flexor reflex threshold using IM versus cutaneous stimulation at the various sites. Consistent differences in flexion reflex latencies were also observed at higher (50 mA) stimulus intensities but were not statistically significant across all stimulus paradigms. In addition, substantial differences in the mean and variability of latency to peak hip reflex torques were also demonstrated with maximal reflex

FIG. 2. Stimulus response relationships after electrocutaneous medial arch/1st web space vs. IM TA stimulation in 6 subjects with flexion reflexes throughout 10- to 50-mA stimulus intensities [mean threshold = 5.58 mA (3.58 times motor threshold)]. Decreased modulation of flexion reflex responses was noted after IM vs. cutaneous stimulation, although no difference in ratio of ankle to hip torque was observed. The latter result indicates that flexor reflexes elicit after IM TA and electrocutaneous were quantitatively similar.

FIG. 3. Flexion reflexes after medial arch/1st web space stimulation (A: gray trace) and IM TA stimulation (A: black trace) vs. electrocutaneous stimuli applied to surface of tibia (B) and TA (C). Differences in latency of onset of flexion reflexes were demonstrated between IM vs. all electrocutaneous stimulation and detailed in Table 1.
torques after IM stimulation appearing after a longer delay than responses after electrotactile stimuli. Significant differences in total flexion reflex duration between the stimulation sites at 2 times threshold or 50-mA stimulus amplitudes were not observed, however (lowest $P$ value >0.10). Mean latency differences between cutaneous and IM stimulation were ~40–60 ms, which is nearly consistent with contraction times for mixed fiber-type muscles in reduced preparations (Burke 1981) and for motor unit and whole muscle contraction times of the TA in individuals with complete SCI (Stein et al. 1992).

**Modulation of stimulus parameters to alter dorsiflexion torque**

To assess the possible contribution of the stimulus-induced muscle torque to the resultant ankle and hip reflex torques, IM TA stimulus parameters of amplitude and frequency were varied independently to modulate the magnitude of TA contraction. Figure 4 shows the stimulus-induced muscle torques at the ankle and the ankle (A) and hip flexion reflex torques (B) for one subject at multiple stimulus amplitudes. For the range of stimuli shown, both the ankle stimulus-induced muscle torque and ankle and hip reflex torques modulate with current amplitude.

A multilevel regression analysis (Bryk and Raudenbush 1992) (please see METHODS) was performed to assess the relation between stimulus parameters (amplitude and frequency of IM stimulation) versus the ankle and hip torques during flexion reflex behaviors and stimulus-induced muscle torques versus reflex torques. Figure 4, C and D, illustrates the results from one subject. Specifically, the linear relationships of the stimulus amplitude and stimulus-induced muscle torque versus ankle and hip reflex torques are shown prior to nesting (i.e., normalization) procedures. As demonstrated here, the stimulus induced motor torque generated at the ankle was correlated to a greater extent to the ankle and hip reflex torques with similar results observed after modulation of stimulus frequency.

For all subjects, regression coefficients for the relationship between stimulus parameters (amplitude or frequency) and ankle and hip reflex torque and those for the stimulus-induced torque versus reflex torques were statistically significant (Table 2). However, regression coefficients were always greater for the stimulus-induced torque versus reflex torque relationships (e.g., during stimulus amplitude variation, regression coefficient for stimulus amplitude vs. hip reflex torque = 0.54, and for stimulus-induced torque vs. hip reflex torque = 0.57, both $P < 0.001$). In addition, when determining the unique contribution (i.e., unique variance) of the stimulus-induced muscle torque to the reflex torques after controlling for the stimulus parameters (amplitude or frequency), all relationships were statistically significant ($P < 0.01$; e.g., unique variance of stimulus-induced torque versus hip reflex torque = 0.16). In contrast, the unique variance of the relationships between the

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**FIG. 4.** Modulation of ankle dorsiflexion torques after direct IM TA stimulation and subsequent flexion withdrawal reflexes. Data for ankle (A) and hip (B) torques during IM TA stimulation and flexion withdrawal shown for 1 subject to illustrate methods for analysis. Data for ankle (C) and hip (D) motor vs. reflex torques (●, —) and current amplitude vs. reflex torques (●●, ——) is demonstrated for same subject, prior to statistical normalization for intersubject comparisons (note: x axes represent same numerical values for both motor torques and stimulus amplitudes and not a ratio of the 2 values). Correlation coefficients were greater for stimulus-induced muscle torques that modulated stimulus variables (amplitude and frequency). Correlation coefficients for grouped data presented in Table 2.

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the nine subjects tested, four generated flexor responses to although to a lesser degree than IM TA stimulation (Fig. 5). Of GS stimulation.

Motor responses after IM plantar flexor stimulation

To assess the excitability of flexion reflex pathways after IM stimulation of other muscles contributing to flexion withdrawal reflexes, we delivered IM stimulation to the GS in 9 of 13 subjects. In contrast to the TA muscle, which is typically the first muscle activated with flexion reflexes, the MG muscle is inconsistently active in individuals with SCI (Hornby et al. 2003; Schmit et al. 2000). As performed in the TA, IM wires were inserted into the muscle bellies of the GS (both medial and lateral heads) and stimulus amplitude varied across a 50-mA stimulus amplitude range. Stimulus-induced muscle torques and flexion reflex ankle and hip torques were determined and compared with those after medial arch and surface GS stimulation.

In general, IM GS stimulation could elicit a flexor reflex, although to a lesser degree than IM TA stimulation (Fig. 5). Of the nine subjects tested, four generated flexor responses to IM GS stimulation. Of those, the mean and SD of threshold for flexion withdrawal across subjects was 16 ± 11 (range: 2–30 mA) for IM stimulation versus 15 ± 7.5 mA (range: 5–30 mA) for medial arch stimulation. For surface GS stimulation, flexion reflexes were elicited in 7/9 subjects, but at a higher current threshold than those elicited at the medial arch (26 ± 12 mA; P < 0.05). Flexion withdrawal thresholds after surface GS stimulation were not, however, significantly different from threshold from IM GS stimulation. Due to the small sample size, it is unclear if there was an inherent difference in the ability to generate flexor responses after GS stimulation as reflexes were elicited consistently in most subjects after electrocutaneous stimuli. The difference in IM sensitivity may be related to a difference in the cross-sectional area of the muscle, which would distribute the load to a greater extent resulting in lower likelihood of activating load-sensitive receptors. Alternately, the lower sensitivity may be a result of the shortened position of the triceps surae complex, thereby generating less torque during IM stimulation (Cleland and Rymer 1990).

**DISCUSSION**

Prolonged flexion reflexes (i.e., flexor spasms) were triggered in individuals with SCI using IM TA and GS stimulation. Peak, isometric torques after IM stimulation were quantitatively similar to flexion reflexes initiated by electrocutaneous stimulation, although the latencies of flexion reflexes after IM stimuli were consistently longer. The mean electrical stimulus intensity required to elicit flexion reflexes after IM stimulation was within the range of direct group Ib-II afferent recruitment and never below motor threshold. Further, ankle dorsiflexion torques after IM TA stimulation (i.e., the stimulus-induced muscle torques) were correlated to a greater extent with ankle and hip reflexes in comparison to the stimulus amplitude or frequency parameters. These findings indicate a possible contribution of muscle afferents toward the initiation of flexion reflexes following SCI. Finally, IM stimulation of the GS also elicited flexion reflexes, although the response was observed in

**TABLE 2. Standardized regression coefficients of stimulus parameters and stimulus-induced muscle torques versus ankle and hip reflex torques**

<table>
<thead>
<tr>
<th></th>
<th>Reflex Ankle Torque</th>
<th>Reflex Hip Torque</th>
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<tbody>
<tr>
<td><strong>Δ Amplitude</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulus parameter (amp)</td>
<td>0.71 (&lt;0.001)</td>
<td>0.54 (&lt;0.001)</td>
</tr>
<tr>
<td>Unique variance</td>
<td>0.14 (&lt;0.01)</td>
<td>0.10 (0.06)</td>
</tr>
<tr>
<td>Stimulus-induced torque</td>
<td>0.79 (&lt;0.001)</td>
<td>0.57 (&lt;0.001)</td>
</tr>
<tr>
<td>Unique variance</td>
<td>0.20 (&lt;0.001)</td>
<td>0.16 (&lt;0.001)</td>
</tr>
<tr>
<td><strong>Δ Frequency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulus parameter (freq)</td>
<td>0.38 (&lt;0.01)</td>
<td>0.23 (&lt;0.01)</td>
</tr>
<tr>
<td>Unique variance</td>
<td>0.07 (0.35)</td>
<td>0.02 (0.83)</td>
</tr>
<tr>
<td>Stimulus-induced torque</td>
<td>0.54 (&lt;0.001)</td>
<td>0.35 (&lt;0.001)</td>
</tr>
<tr>
<td>Unique variance</td>
<td>0.36 (&lt;0.001)</td>
<td>0.26 (&lt;0.01)</td>
</tr>
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Standardized regression coefficients (beta weights) and P values from the multilevel regression analysis are provided to indicate relationships between the independent variables of stimulus parameters and stimulus-induced muscle torques versus the dependent variables of resultant ankle and hip reflex torques. Data are grouped under the stimulus parameter conditions of amplitude and frequency. For each stimulus parameter, the standardized regression coefficients for the relations between the two stimulus parameters versus ankle and hip reflex torques and stimulus-induced torques versus reflex torques are provided. The regression coefficients are interpreted conventionally with P values provided in parentheses indicating statistical significance. In addition, the unique contributions (i.e., unique variances) of each independent variable (stimulus parameter or stimulus-induced torque) to the ankle and hip reflex torques were calculated when the other independent variable are accounted for (as described in METHODS), with P values provided. Regression coefficients were always higher for stimulus-induced torques versus reflex torques relationships as compared to stimulus parameters versus reflex torques and statistically significant when the unique variances of the contribution of stimulus-induced torques to reflex torques were calculated. In contrast, the unique contributions of the stimulus parameters (amplitude of frequency) to ankle and hip reflex torques were not significant in most cases.

stimulus parameters versus reflex torques were often not statistically significant when the contributions of stimulus-induced muscle torques were controlled for (unique variance of stimulus amplitude vs. hip reflex torque = 0.10, P = 0.06; please see Table 2). The data demonstrate that the stimulus-induced motor torques contributed to a greater extent to the reflex torque than the stimulus parameters of amplitude or frequency, suggesting a prominent role of the muscle force generated during the IM stimulus mediating the magnitude of the ensuing flexion reflex.

**FIG. 5.** A representative example of EMG and joint torque patterns after IM GS stimulation and flexor reflex activity are demonstrated. Shown are the TA and MG EMG activity after the 200-ms stimulus train, with joints torques from the ankle, knee, and hip. During IM GS stimulation, ankle plantarflexion and knee flexion torques are increase. After termination of the stimulus, the onset of TA EMG activity and ankle dorsiflexion, and hip flexion indicate the flexion reflex response.
less than half of the subjects. Identification of the specific reflex mechanisms underlying the prolongation of spastic motor behaviors may provide a basis for interventions to alleviate spasticity after neurological injury.

**Reflex initiation by cutaneous or muscle afferent stimulation versus muscle contraction?**

Identification of potential afferent mechanisms underlying generation of flexion reflexes in human SCI after IM stimulation is necessarily indirect, although candidates include direct stimulation of muscle or cutaneous afferents or indirect activation of flexor reflex afferents after muscle contraction and force generation. Two findings presented here provide an indication that the stimulus-induced muscle contraction played a substantial role. First, flexor reflex torques generated after modulation of IM TA stimulus parameters were correlated to a greater extent with the stimulus-induced muscle torque than to the stimulus parameters (Fig. 4 and Table 2). If direct afferent stimulation was the primary source of flexion reflex initiation, flexion reflexes should modulate according to the amplitude and frequency of stimulation. Rather, the unique variance of the linear relationships between the stimulus parameters and reflex torques was always less than that of stimulus-induced muscle torques versus reflex torques and often not significant. It remains possible that direct muscle afferent stimulation may contribute to flexion reflex responses, although in the present study, a substantial component of the reflex torques was attributable to the stimulus-evoked force.

Another consistent finding supporting the notion that stimulus-induced muscle contraction was a critical stimulus for flexion reflex initiation was the delayed latency of the onset of flexion reflex after IM TA versus cutaneous stimulation. Further, significant increases in the latency to maximum hip torques after IM stimuli, with substantial variability in the grouped response, were observed. While both IM and cutaneous stimuli recruit a variety of afferents of variable diameter, flexion reflexes generated by direct activation of muscle afferents would be expected to have a similar latency to electrophysiologically applied stimuli. Rather, the difference in reflex latencies after IM and electrophysiological stimulation was ∼40–60 ms, consistent with contraction times of human TA muscle after complete SCI (Stein et al. 1992), indicating that the stimulus-evoked muscle contraction may have played a role in flexor reflex initiation.

Similarly, significantly greater mean and variability of latencies of maximum hip torques were also observed after IM TA stimulation. If the stimulus-induced muscle contraction is indeed a primary stimulus for the delayed flexion reflexes, greater latencies of maximal hip torques may be partially explained by the slow decay of stimulus-induced muscle contraction, which could prolong the force-mediated input to flexor reflex pathways. Because the precise mechanisms required for reflex initiation and habituation after direct muscle excitation are incompletely understood and may involve multiple mechanisms (e.g., direct vs. indirect muscle afferent stimuli), the large variability of maximum hip torque latencies is not entirely unexpected. The findings of delayed latencies for onset and maximum hip torques do, however, provide some evidence for the contribution of muscle afferents to prolonged flexion reflexes after SCI.

**Afferent mechanisms of flexor spasms with IM stimulation**

If the stimulus-evoked muscle contraction triggers flexor reflexes after SCI, multiple muscle afferent pathways could potentially be responsible. In previous studies on individuals with chronic SCI, imposed ankle rotation was shown to initiate multi-joint flexion reflexes (Schmit et al. 2000, 2002). In particular, the amplitude of flexion reflexes was unrelated to the velocity of ankle movement but rather to the joint angle and passive ankle loading at the end range of motion. Such behaviors were not likely to be elicited by Ia afferent input, but possibly through receptors responsive to either muscle length (group II), muscle force (group Ib, III-IV), or cutaneous and/or joint afferent input. In preliminary experiments on three subjects with SCI, reduction of cutaneous afferent input after anesthesia of cutaneous nerves supplying the foot did not alter the magnitude of movement triggered flexion reflexes (Schmit, Hornby, and Benz, unpublished results). The results indicate that muscle and/or joint, but not cutaneous afferents, play a prominent role in movement-triggered flexion reflexes.

Considering the depth of IM fine wire insertion and consistent reflex delay, the most likely candidates for flexion reflex initiation in our studies include both length- and force-sensitive muscle versus cutaneous afferents. While direct recruitment of group II spindle afferents can elicit flexion reflexes (Eccles and Lundberg 1959), modulation of group II discharge from secondary muscle spindles is relatively limited during isometric conditions (Edin and Vallbo 1990; Prochazka 1990). Further, the high correlation coefficients of stimulus-induced muscle torques to peak reflex torques indicate that force-sensitive afferents would more than likely figure prominently in flexion reflex generation.

Potential candidates for load-sensitive afferent pathways mediating flexor reflexes after stimulus-induced muscle contraction include the Ib afferent projections and group III–IV pathways from muscular free nerve endings. While the role of Golgi tendon organs cannot be completely excluded, excitatory
Ib inputs have been implicated primarily during locomotor tasks in extensor (Pearson and Collins 1993) and possibly flexor (Quevedo et al. 2000) motoneurons (reviewed in Dietz and Duysens 2000). During static, nonpostural tasks, such afferent input is typically inhibitory to motoneuron pools. Further, recruitment of Ib afferents at the mean electrical stimulus strength used in this study would occur during both direct electrical and indirect mechanical stimulation, the latter as a result of the stimulus-induced contraction. The long latency of the flexor reflexes indicate that recruitment of afferent pathways other than those that could be directly stimulated at the lower current amplitude were responsible for the observed reflex behavior. Group III–IV afferents from muscular free nerve ending therefore serve as an attractive explanation.

Previous evidence has indicated a role for force- and stretch-sensitive interneurons in the generation of clasp-knife behaviors in reduced preparations (Cleland and Rymer 1990; Cleland et al. 1982, 1990). Specifically, in the decerebrate cat after dorsal spinal hemisection, flexion reflexes elicited by increases in passive stretch or muscle loading (Cleland and Rymer 1990) are analogous to behaviors seen in human SCI as demonstrated in the present and previous studies (Schmit et al. 2000, 2002). Specifically, the clasp-knife reflex, which consists of brief stretch-triggered excitation of the extensors followed by long-lasting inhibition, is qualitatively similar to the response of the IM GS stimulation in the present study. Muscular group III–IV (and nonspindle group II afferents) were thought to play a prominent role in triggering flexion reflexes during imposed movements in human SCI (Schmit et al. 2000, 2002) as these afferents respond to changes in both muscle length and force. Further, in contrast to Ib and spindle II afferents, activity of group III–IV afferents are tightly correlated to the clasp-knife inhibition/flexion reflex behaviors (Cleland et al. 1990).

In combination with previous findings in reduced animal preparations and individuals with SCI, our experimental results suggest that changes in lower extremity flexor or extensor muscle length, or passive loading via electrical or mechanical stimuli, can elicit flexor spasms after chronic SCI (Schmit et al. 2000, 2002). Flexion reflexes were prevalent in most subjects after IM TA stimulation and in some cases after GS stimulation. Differences in elicitation of flexion reflexes between muscle groups may be due to the lower extremity posture in which knee flexion and ankle plantarflexion may render the GS muscle with decreased muscle length and hence passive or active loading during IM GS stimulation to generate forces necessary to elicit flexion reflexes. Future work will investigate whether changes in muscle loading of plantar- and dorsiflexor muscle groups during electrical stimulation at various muscle lengths to alter flexor reflex behavior after electrical stimulation.

Role of central pathways to reflex behaviors after IM stimulation

The precise cellular mechanisms underlying hyper-excitability of flexion reflex pathways in human SCI are not known. One prominent theory is that spinalization results in the loss of descending neuromodulatory input, thereby releasing afferent and interneuronal (particularly dorsal horn) pathways from inhibition (Engberg et al. 1968; Heckman 1994). Increases in excitability are likely demonstrated as release of presynaptic inhibition of primary afferents (Garraway and Hochman 2001) or alterations in passive cellular properties (Jankowska 1992). Manifestation of active (discharging) behaviors such as plateau potentials (Hornby et al. 2003) or central oscillatory circuits (Beres-Jones et al. 2003) may also contribute to prolonged flexion reflexes. Whether exaggerated flexor reflexes are due to alterations in afferent and interneuronal pathways alone or in combination with changes in flexor motoneuron behavior after chronic spinalization (Bennett et al. 2001a,b) remains unknown. Our previous (Hornby et al. 2003) and current data suggest that both cellular and reflexive mechanisms may contribute to prolonged flexor spasms and are not mutually exclusive.

Potential significance to manifestation of prolonged flexor spasms

The current findings indicate a contribution of the muscle load afferents to the prolongation of flexor spasms in human SCI. Activation of load-sensitive afferents, which likely occurs during spastic motor behaviors, may provide additional excitation of flexion reflex pathways. For example, a flexion reflex may be initiated from cutaneous, joint, or muscular stimuli, resulting in muscle contraction that, in turn, activates load-sensitive muscle afferents causing further excitation of the flexion reflex pathways. Such self-reinforcing reflexes have been implicated in the maintenance of extensor (e.g., Conway et al. 1987; Hiebert and Pearson 1999; Pearson and Collins 1993; for review, see Dietz and Duysens 1993) and more recently, flexor (Quevedo et al. 2000) muscle activity during locomotion, and in extensor motor pools during static stance (Pratt 1995) in feline preparations. Further, positive force feedback has been implicated in the regulation of extensor motor activity during the stance phase of gait (Harkema et al. 1997) or static postural tasks in humans (Dietz et al. 1992).

Evidence for positive feedback prolongation of spastic motor behaviors after neurological injury has not been established, however. Despite the possibility of afferent regulation of extensor activity during lower extremity weight bearing, low-eccentric-threshold, load-sensitive afferents are typically inhibitory during static, non-weight-bearing conditions (Eccles et al. 1957; Pearson and Collins 1993). The requirements for positive force feedback control are, however, only that the presumed input to the flexor reflex circuitry is excitatory and that this circuitry is sufficiently hyperexcitable to enhance flexor activity. Both conditions appear to be satisfied in both experimental animal (Cleland et al. 1982, 1990) and human conditions (Hornby et al. 2003) after SCI. With the reflex gain of the system less than unity (Prochazka 1996), or at higher reflex gains and sufficient delay in the reflex pathways (Prochazka et al. 1997), positive force-feedback control could result in reinforced flexor muscle activity. Although central mechanisms certainly may play a role in spasms after SCI (Beres-Jones et al. 2003; Hornby et al. 2003), the contribution of afferent regulation of spastic motor behavior requires further investigation.

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