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Tail Muscles Become Slow but Fatigable in Chronic Sacral Spinal Rats With Spasticity

R. Luke W. Harris, Jacques Bobet, Leo Sanelli, and David J. Bennett

Centre for Neuroscience, University of Alberta, Edmonton, Canada

Abstract

Paralyzed skeletal muscle sometimes becomes faster and more fatigable after spinal cord injury (SCI) because of reduced activity. However, in some cases, pronounced muscle activity in the form of spasticity (hyperreflexia and hypertonus) occurs after long-term SCI. We hypothesized that this spastic activity may be associated with a reversal back to a slower, less fatigable muscle. In adult rats, a sacral (S₂) spinal cord transection was performed, affecting only tail musculature and resulting in chronic tail spasticity beginning 2 wk later and lasting indefinitely. At 8 mo after injury, we examined the contractile properties of the segmental tail muscle in anesthetized spastic rats and in age-matched normal rats. The segmental tail muscle has only a few motor units (<12), which were easily detected with graded nerve stimulation, revealing two clear motor unit twitch durations. The dominant faster unit twitches peaked at 15 ms and ended within 50 ms, whereas the slower unit twitches only peaked at 30–50 ms. With chronic injury, this slow twitch component increased, resulting in a large overall increase (>150%) in the fraction of the peak muscle twitch force remaining at 50 ms. With injury, the peak muscle twitch (evoked with supramaximal stimulation) also increased in its time to peak (+48.9%) and half-rise time (+150.0%), and decreased in its maximum rise (–35.0%) and decay rates (–40.1%). Likewise, after a tetanic stimulation, the tetanus half-fall time increased by 53.8%. Therefore the slow portion of the muscle was enhanced in spastic muscles. Consistent with slowing, posttetanic potentiation was 9.2% lower and the stimulation frequency required to produce half-maximal tetanus decreased 39.0% in chronic spinals. Interestingly, in spastic muscles compared with normal, whole muscle twitch force was 81.1% higher, whereas tetanic force production was 38.1% lower. Hence the twitch-to-tetanus ratio increased 104.0%. Inconsistent with overall slowing, whole spastic muscles were 61.5% more fatigable than normal muscles. Thus contrary to the classical slow-to-fast conversion that is seen after SCI without spasticity, SCI with spasticity is associated with a mixed effect, including a preservation/enhancement of slow properties, but a loss of fatigue resistance.

INTRODUCTION

Spinal cord injury (SCI) has long been recognized to reduce the level of neuromuscular activity in skeletal muscles innervated below the level of the lesion and thus alter their physiological and histological properties (Shields 2002; Talmadge et al. 1995). Such a decrease in neuromuscular activity typically results in atrophy of skeletal muscles and slow-

to-fast transformation in myosin heavy chain isoform expression (Burnham et al. 1997; Lieber et al. 1986a; Talmadge et al. 1995). These changes render muscles smaller, faster, weaker, and more fatigable (Lieber et al. 1986b; Talmadge et al. 2002). These effects occur in all hindlimb muscles but are somewhat larger in predominantly slow muscles such as the soleus (Davey et al. 1981; Lieber et al. 1986a,b). Importantly, interventions such as functional electrical stimulation and exercise have been found to minimize (counteract) such changes or restore muscle properties to normal (Hartkopp et al. 2003; Kernell et al. 1987a,b; Rochester et al. 1995; Roy et al. 1998, 1999).

In humans and a few animal models, SCI results in a spasticity syndrome that includes hyperreflexia, hypertonus, and muscle spasms (Bennett et al. 2004; Fujimori et al. 1968; Heckman 1994; Kuhn and Macht 1948; Lance and Burke 1974; Ritz et al. 1992; Taylor et al. 1997). This spastic neuromuscular activity after SCI should, in principle, result in activity-dependent modification of muscle contractile properties (slowing and improved resistance to fatigue), just like exercise training or electrical stimulation does, as described above. However, contractile properties have mainly been studied in animal models of chronic SCI, where no clear spasticity develops (Davey et al. 1981; Roy et al. 1998, 1999) or where spasticity in the muscle studied is not reported in detail (Lieber 2002; Lieber et al. 1986b; Mayer et al. 1984). Thus an important question that remains is how spastic neuromuscular activity influences the properties of muscles innervated below the level of a spinal cord lesion.

The segmental tail muscles of adult rats develop a clear spasticity syndrome, with pronounced spasms, beginning 2 wk after a complete transection at the sacral level (Bennett et al. 2004). This tail spasticity resembles that observed in human individuals with long-term SCI (Bennett et al. 1999, 2004) and can be detected in segmental tail motor units of awake chronic spinal rats in the form of prolonged muscle spasms that last many seconds, and hypertonus associated with motor unit firing that lasts from minutes to hours (Bennett et al. 2001). Recently, we have performed 24-h intramuscular EMG recordings revealing a dramatic reduction in muscle activity with acute injury compared with normal, and a recovery to approximately normal activity levels caused by spasms (active whenever the animal moves or tail contact is made) after chronic injury with spasticity (unpublished data). Thus in this study, we examined the contractile properties of whole segmental tail muscles in vivo in the same chronic sacral SCI rats used previously to characterize reflex behavior and the time-course of the development of spasticity (Bennett et al. 2004) and in uninjured control rats. We hypothesized that the overall muscle contractile speed and fatigability should decrease or at least be preserved in spastic muscles, compared with normal, in support of the idea that pronounced spastic muscle activity can counter the classic effects of reduced activity caused by paralysis after SCI (conversion to more fatigable, faster properties), described above (Shields 2002; Talmadge et al. 1995). Parts of this work have previously been reported in abstract form (Stephens et al. 1999).

METHODS

Spinal cord transection

A complete spinal cord transection was made at the S₂ sacral level in female Sprague-Dawley rats at ~2 mo of age (adult rats) under pentobarbital sodium anesthesia (58 mg/kg body mass). These rats were drawn from a population of rats used in an earlier study (Bennett et al. 2004) and within 2–3 mo, as previously documented, dramatic spasticity developed in the tail muscles and continued indefinitely (mean spasticity rating = 4/5; see Bennett et al. 1999, 2004 for details of the chronic spinal cord transection and spasticity assessment). At ~8 mo after injury (mean age, 10.0 mo; $n = 5$), the contractile properties of muscles from chronic spinal rats with spasticity were studied and compared with those of muscles from age-matched control rats (mean age, 9.7 mo; old normal rats, $n = 5$) and from younger control rats close to the age at which the transection was made (mean age, 3.0 mo; young normal rats, $n = 8$; see Table 1). The latter group was used to control for the possibility that the normal changes in muscle properties with age may be arrested by SCI (Talmadge et al. 1995). However, there was no reason to expect, a priori, that a large age effect would be observed between animals at the transection age and normal animals at the final age of the chronic spinal rats, because aging studies have revealed few differences in muscle contractile properties between adult animals of these ages (Ansved and Larsson 1989). All experimental procedures were carried out according to guidelines of the Canadian Council for Animal Care and with the approval of the University of Alberta Animal Welfare and Policy Committee.

Tail muscle and nerve preparation for recording

For recording muscle properties, chronic spinal rats with spasticity and young and old normal rats were anesthetized with 58 mg/kg pentobarbital sodium, the dose being topped up as required. A longitudinal incision ~8 cm long was made in the skin on the left ventrolateral side of the tail, from the 14th caudal vertebra (Ca₁₄) to the base of the tail near the origin of the caudal nerve trunk supplying the muscle. The 14th ventrolateral segmental muscle was exposed, and the fascia covering the muscle was cleared, taking care not to damage the dorsolateral vein. The distal end of the muscle was gently separated from the underlying bone and connective tissue with fine forceps. 3–0 surgical silk was threaded under the muscle and securely knotted around the tendon at the muscle's distal tip. To ensure that the suture did not slip, the knot was also glued (RP 1500, super glue, Adhesive Systems, Frankfort, IL). The muscle and tendon were cut completely from the bone at that end. The 3–0 suture was connected at its loose end to a combination muscle puller/force transducer (Cambridge Technologies model 300B servomotor). At the base of the tail, the nerve supplying the Ca₁₄ segmental muscle was freed for 3–5 cm. The entire tail, including the exposed nerve and muscle, was bathed in mineral oil, with the skin flaps around the incision pinned back to a silicone epoxy base at the bottom of the bath, and maintained at 35°C with radiant heat. The nerve was placed across the two leads of a bipolar stimulating electrode connected to a variable rate and voltage stimulator (Grass Instruments SD10), and the nerve was crushed with forceps 2 mm rostral to the electrode. The stimulation intensity was usually set supramaximal to a full muscle twitch, unless otherwise stated in RESULTS.

Contractions

Initially, the muscle was lengthened slowly during repeated maximal twitch contractions until maximal isometric twitch force was produced, and contractile properties were recorded with the muscle set at this optimal length. Any force present at rest at this length was assumed to be passive and was subtracted from all subsequent trials. Force was sampled at 1,000 Hz using a digital data acquisition system (Axoscope, Axon Instruments, Union City, CA). Evaluation of muscle contractile properties was performed with a broad range of contractions including single supramaximal twitches, tetani evoked by 2-s supramaximal stimulus trains at 200 Hz, twitches before and after a brief fused tetanus (200 Hz), and unfused and fused tetani at stimulation frequencies of 10–200 Hz. Additionally, we inferred motor unit twitch responses using discrete, stepwise increments in stimulus intensity, as detailed in RESULTS. To assess muscle fatigability, the muscle was stimulated supramaximally at 40 Hz for 300 ms once per second for 100 s (Burke et al. 1973). The fatigue index was calculated as the ratio of the peak force in the 100th second to the peak force in the 1st second, where both these values were measured relative to the baseline force before the 1st contraction.

Analyses

Analyses of contractions were performed with Clampfit (Axon Instruments, Union City, CA), SigmaPlot (SPSS, Chicago, IL), Excel (Microsoft, Redwood, CA), and custom programs prepared with MATLAB (MathWorks, Natick, MA). All data are reported as group means and SD. A Student's *t*-test was used to determine statistical significance at the 95% confidence level ($P < 0.05$).

RESULTS

Body and muscle masses of normal and chronic spinal rats

Both chronic spinal rats and age-matched old normal rats had significantly greater total body mass than did young normal rats (Table 1). The wet mass of the tail muscles from chronic spinal rats was significantly less than old normal rats, but not different from young normal rats (Table 1). Tail muscle mass was not significantly different between young and old normal rats when normalized to body mass (muscle-to-body mass ratios), but chronic spinal rats had muscle-to-body mass ratios that were significantly lower than in young and old normal rats (27.7% less than old normal rats; Table 1). As differences in body size are accounted for in this ratio, the change in relative mass reflects considerable atrophy of the tail muscles after injury.

Tail muscles of chronic spinal rats have slower and larger twitches than tail muscles of normal rats

The tail muscle twitch force generated during a supramaximal electrical stimulation of the nerve is shown for a typical old normal rat with dashed lines in Fig. 1, *A* and *B*. Young normal rats and old normal rats were not significantly different in either time to peak twitch (Fig. 1 *C*) or mean half-rise time (Fig. 1 *D*). In contrast, in chronic spinal rats, supramaximal nerve stimulation evoked twitches that were substantially prolonged relative to those in old

normal rats (Fig. 1, *A* and *B*, solid lines). That is, the time to peak twitch in chronic spinal rats was significantly longer than in young and old normal rats (48.9% larger than old normal rats; Fig. 1*C*), as was the half-rise time (150.0% larger than old normal rats; Fig. 1*D*).

Young normal rats had, on average, 32.7% lower twitch force than old normal rats (significantly lower peak twitch; Fig. 1*E*), as expected for a much smaller muscle (Table 1). However, the twitch force normalized to the muscle mass was not significantly different between young and old normal rats (Fig. 1*F*). Interestingly, the absolute peak twitch force was significantly larger in spastic muscles than in normal muscles (85.2 and 39.5% greater, respectively, than young and old normal rats; Fig. 1*E*). Furthermore, twitch force normalized to muscle mass was almost doubled in spastic muscles from chronic spinal rats compared with muscles from young normal rats (88.0% increase, significant difference) and old normal rats (81.1% increase, significant difference; Fig. 1*F*). Thus spastic muscles contracted more slowly and generated more force in a twitch.

Motor unit twitches were enhanced in chronic spinal rats

Discrete, stepwise increases in stimulus intensity elicited large, discrete muscle twitch force increments (e.g., from *a* to *b* in Fig. 2, *A* and *B*) that were the result of the recruitment of new units. Thus by subtracting the means of successive, discrete twitch levels as the force increased, it was possible to infer the twitches of individual motor units (Tam et al. 2001). No doubt some of these subtractions may have contained multiple motor unit twitches, but given that these muscles have 12 or fewer motor units (Steg 1964), most subtractions likely reflected a single motor unit twitch (Fig. 2; see also Tam et al. 2001). The low number of motor units in the segmental tail muscles made this analysis possible, despite occasional motor unit alternation (Wang et al. 2004). When stimulating the nerve, two factors determine the recruitment order: the size and the random distribution of axons in the nerve. Because of this random element, the closest axons rather than the largest were often recruited first, and thus the larger, faster units were not always recruited first (e.g., *a* in Fig. 2, *A* and *B*).

These motor unit profiles revealed fast and slow motor unit twitches in both normal and injured animals. The fastest motor unit twitches made up the majority of the force in both normal and chronic spinal animals, with a time to peak of ~15 ms (Fig. 2, *A* and *B*), consistent with previous findings (Steg 1964). Conveniently, these fast twitches ended almost completely within 50 ms, whereas the slower motor unit twitches had a time to peak force of ~30 ms in normal rats and ~50 ms in chronic spinal rats.

Thus the twitch force measured at 50 ms and normalized to peak force (TT_{50}) was a useful measure of the total contribution of the slower motor units to the twitch contraction (Fig. 2), because the major fast component had ended by that time. This TT_{50} was not significantly different between young normal rats and old normal rats (Fig. 2*C*). However, TT_{50} in chronic spinal animals was significantly greater on average than in both young normal rats (137.3% larger) and old normal rats (153.4% larger; Fig. 2, *A–C*). This tripling of TT_{50} in whole muscle after injury suggests an overall slowing of some of the motor units that contribute to the twitch in spastic tail muscles.

Absolute rate of rise of twitch force is not lower in chronic spinal rats, it just takes longer to develop the larger peak force

The absolute rates of rise (Fig. 3*A*) and decay (Fig. 3*B*) of twitch force (determined from the derivative of the twitch) for chronic spinal rats were not significantly different from those for young or old normal rats. However, we normalized twitch rate to peak twitch force, and this normalized maximum rate of rise of twitch force in chronic spinal rats was significantly lower than in normal rats (35.0% lower in chronic spinal rats than in old normal rats; Fig. 3*C*), as was the normalized maximum rate of twitch force decay (40.1% lower than in old normal rats; Fig. 3*D*). This was simply caused by the longer-lasting rise and fall of the twitch in spastic tail muscles, because it rose to a larger peak amplitude at the same absolute speed. These results may be consistent with reduced rates of buffering and sequestration of free myoplasmic calcium after stimulation, allowing longer periods of Ca^{2+} availability and thus greater, longer force production in chronic spinal rats (see DISCUSSION).

Tetani and posttetanic twitch properties of tail muscles

Despite the larger twitches seen after chronic sacral SCI, the absolute peak tetanic force (supramaximal stimulation at 200 Hz) was significantly reduced in spastic muscles relative to that in old normal control muscles (38.1% smaller in chronic spinal rats than in old normal rats; Fig. 4, *A–C*). However, the peak tetanic force in chronic spinal rats was not significantly reduced relative to that in young normal rats (Fig. 4*C*). When normalized to muscle mass to account for changes caused by muscle atrophy, the tetanic force was not significantly different among tail muscles of chronic spinal rats, old normal rats, and young normal rats (Fig. 4*D*). Importantly, tetanus half-rise time was not significantly different across groups (Fig. 4*E*), but the tetanus half-fall time was significantly prolonged in spastic muscles relative to normal muscles (53.8% longer than in old normal rats; Fig. 4*F*).

Segmental tail muscle twitches in both normal and chronic spinal animals were potentiated immediately after tetani (relative to the prior twitches; Fig. 4, *A*, *B*, and *G*). This posttetanic potentiation was not significantly different in young normal rats and old normal rats (Fig. 4*G*). Twitches of chronic spinal rats' tail muscles potentiated significantly less than did those of young and old normal rats (9.2% lower posttetanic potentiation (PTP) than in old normal rats; Fig. 4*G*), consistent with a transition toward slower muscle properties, because slower muscles typically have lower posttetanic potentiation (Davey et al. 1981), or even exhibit posttetanic depression of twitch (Stephens and Stuart 1975).

Young normal rats had twitch-to-tetanus ratios that were not significantly different from old normal rats (4.3% difference; Fig. 4*H*). The twitch-to-tetanus ratio was much larger in chronic spinal rats than in young and old normal rats (chronic spinal rats 104.0% larger than old normal rats, significant difference; Fig. 4*H*), as similarly observed by Lieber et al. (1986b) in paralyzed hindlimb muscles after SCI compared with normal muscles. Thus relative to tetani, chronic spinal tail muscles have larger twitches than do normal rats, consistent with the increased twitch force but decreased tetanic force observed here.

Chronic spinal tail muscles fuse more easily

When the tail nerve was stimulated with 300-ms trains of increasing frequencies (10–200 Hz), the tetanic muscle force production was initially low and unfused at low frequencies, and became larger and more fused at higher stimulation frequencies. When the tetanic force production at each frequency was normalized to the maximum, fused tetanic force (at 200 Hz), on average, this normalized tetanic force was nearly identical at all frequencies in young and old normal rats (not significantly different; ○ and □ overlay in Fig. 5A). However, the normalized tetanic force production was significantly larger in chronic spinal rats (Fig. 5A, ●) at all frequencies 70 Hz compared with in young and old normal rats. Also, the stimulation frequency required to achieve the half-maximal tetanic force, F_{50} , was significantly lower for muscles of chronic spinal rats compared with those of normal rats (39.0% lower; Fig. 5B). Thus in chronic spinal rats with spasticity, tetani became fused and reached maximum tetanic force production at lower frequencies than in normal rats. This is in contrast to the higher fusion frequency observed for nonspastic hindlimb muscles after SCI (Lieber et al. 1986b) but again is consistent with a slowing of the spastic muscles.

Chronic spinal tail muscles are fatigable

Fatigability of segmental tail muscles was measured by producing 300-ms tetanic contractions (each with supramaximal stimulation at 40 Hz), repeated once per second for 100 s (see METHODS and Fig. 6). Overall, during these repeated contractions, the muscles of chronic spinal rats, compared with normal rats, reached a maximum tetanic force after fewer contractions (~9 contractions in chronic spinal rat, shown in Fig. 6B, *inset*, compared with ~17 contractions in old normal rat, shown in Fig. 6A, *inset*). However, the tetanic force dropped more quickly (after fewer contractions) and reached a lower steady-state tetanic force by the 100th contraction (Fig. 6, A and B, *inset*), suggesting that the chronic spinal muscles were more fatigable.

To quantify the fatigability, the fatigue index was calculated as the ratio of the maximum steady-state tetanic force produced in the 100th contraction to the maximum steady-state tetanic force produced in the 1st contraction (both forces were measured relative to the baseline force before the 1st contraction; see Fig. 6, A and B). On average, the fatigue index was not significantly different in young and old normal rats (Fig. 6C). However, the fatigue index was significantly lower in chronic spinal rats than in young (71.5% lower) and old (61.5% lower) normal rats (Fig. 6C). Thus the muscles of chronic spinal rats were more fatigable than were those of normal rats.

In tail muscles from spastic animals relative to those from normal animals, there was also a much greater rise in the baseline force before the 100th contraction, indicating that the muscle remained more contracted between contractions. This rise in baseline force is consistent with contractures resulting from compromised metabolic capacity (Layzer 1986). This larger baseline rise in chronic spinal rats tended to increase the force measured on the 100th contraction and thus resulted in an underestimation of muscle fatigue as measured by the fatigue index.

DISCUSSION

These results show that, in rats after chronic sacral SCI and spasticity, the segmental tail muscles undergo substantial changes in contractile properties. We suggest that some of these changes result from an increase in muscle activity caused by the prominent spasticity in these muscles (Bennett et al. 2004); that is, there is a slowing of the twitch, a marked increase in peak twitch force, a slowing of relaxation from tetanus, a decrease in fusion frequency, and a decrease in posttetanic potentiation that are all consistent with the contractile phenotype of a classically slower muscle (Davey et al. 1981; Lieber et al. 1986b; Stephens and Stuart 1975). This slowing is similar to that seen in general with increased activity (Hartkopp et al. 2003; Roy et al. 1998, 1999), and thus is consistent with the idea that the spastic activity influences muscle contraction and relaxation, and likely exerts its influence especially on the low-threshold motor units that are more often very active in spasms. In contrast, these muscles of spastic rats also become more fatigable, produce less tetanic force, have increased twitch-to-tetanus ratios, and are atrophied in mass.

Spastic muscle activity depends on the model and muscle examined

The finding that spasticity is associated with slower muscle contractile properties is particularly striking compared with SCI muscle in other studies where spasticity is not observed (Davey et al. 1981; Lieber et al. 1986b; Roy et al. 1998, 1999), again suggesting that spasticity promotes slower muscle contractile properties. That is, after complete spinal cord transections that do not lead to spasticity, there is a classical slow-to-fast myofiber type conversion and muscle atrophy, which result in a faster, more fatigable, and weaker muscle. In these classical studies, the slow type I and fast fatigue resistant type IIA myofibers are markedly reduced and converted to fast fatigable type IID/X and type IIB myofibers (Burnham et al. 1997; Lieber et al. 1986a; Talmadge et al. 1995). Thus the slowing and increased twitch size that we see in spastic tail muscles compared with in normal tail muscles is even more remarkable compared with in nonspastic muscles after SCI, which have faster, weaker contractile properties than normal.

It might be argued that the tail muscles are peculiar in their response to SCI and activity-related changes (spasticity), especially considering that we did not examine the impact on contractile properties of SCI without spasticity. Indeed, several comprehensive studies of the soleus or medial gastrocnemius in cats with complete spinal cord transections are in contrast to our own findings, showing clear slow-to-fast transformations in contractile speed and myofiber type in conjunction with increased fatigability (Cope et al. 1986; Mayer et al. 1984; Munson et al. 1986). Unfortunately, however, these studies either do not report spasticity (Cope et al. 1986; Munson et al. 1986) or do not report in detail the degree of spastic muscle activity (Mayer et al. 1984) in the specific muscles examined, and thus the conclusions they draw regarding mechanisms underlying spasticity must be treated with caution.

In contrast, the tail muscles studied in this paper have been well documented to be spastic (Bennett et al. 2004) and, in particular, the same rats were used in this study as in the study of Bennett et al. (2004). Furthermore, our recent unpublished 24-h EMG recordings indicate that tail muscle activity drops dramatically with acute injury compared with normal, and

with long-term injury and spasticity muscle activity recovers to as much as in the normal rats (although always linked to spasms as opposed to voluntary movements). Thus it is likely that the changes in contractile properties seen in this paper are related to these changes in spastic muscle activity. Furthermore, similar changes in muscle properties (slowing with increased fatigability) have been seen in human muscles that are spastic after long-term SCI (Hidler et al. 2002; Thomas 1997; Zijdwind and Thomas 2003), and thus the changes we see are not peculiar to the rat tail muscle. Moreover, our recent immunohistochemical findings (Harris et al. 2005) reveal that, after SCI, spasticity indeed plays a central role in regulating myofiber type. Specifically, early after injury, tail myofibers identified by immunohistochemistry atrophy dramatically and undergo a transformation toward a faster, more fatigable myofiber distribution, and these changes occur in association with flaccid paralysis, before the development of full spasticity, consistent with other studies of SCI without spasticity (Lieber et al. 1986a; Talmadge et al. 1995). Furthermore, with long-term full spasticity (as in chronic spinal rats in this paper), the opposite occurs: myofibers hypertrophy and undergo a recovery toward slower isoforms (Harris et al. 2005). All myofibers recover completely from atrophy, with the exception of the dominant type-IIID/X myofibers, which partially recover (Harris et al. 2005), consistent with the total muscle mass atrophy described here, and consistent with the idea that the higher threshold units recruited less frequently by spasticity should exhibit fewer activity-dependent adaptations. Ultimately, after long-term injury and spasticity, myofiber size and proportions recover nearly completely to normal (Harris et al. 2005). Consequently, spasticity does indeed preserve myofiber properties over the long term after SCI by countering early atrophy and transformations caused by reduced activity, ultimately normalizing the myofiber phenotype and morphology.

In the discussion below, we suggest possible mechanisms that might explain these changes in contractile properties seen in spastic muscles. As we have just mentioned, with long-term injury and spasticity, the myosin heavy chain-identified myofiber distribution is well preserved compared with normal (Harris et al. 2005) and therefore does not account for the slowing of the muscle that we have observed with long-term injury. Thus we explore the hypothesis that the prolonged and larger twitches seen in these muscles might be caused by decreased intracellular calcium buffering and sequestering that is known to be influenced by increased activity and is associated with longer and larger twitches (Green et al. 1984; Schwaller et al. 1999).

Myofiber type changes with injury

Rat segmental tail muscles have been studied in detail previously only by Steg (1964), who assessed some mechanical and electrophysiological properties of their motor units. The motor unit composition in segmental tail muscles reported by Steg (1964) was ~67% fast, 25% intermediate, and 8% slow. Our recent data from immunohistochemical staining of myosin heavy chain isoforms are consistent with this distribution. We have found that rat segmental tail muscles are composed of predominantly fast myofiber types: fatigue intermediate type-IIID/X myofibers constitute ~65–70% of the muscle; the remaining myofibers are slow nonfatigable type-I (10–20%) and fast fatigue resistant type-IIA (10–20%), and there is a near absence of fast fatigable type-IIB myofibers (Harris et al. 2005).

With long-term SCI, we found that spastic tail muscles exhibit very few changes in myofiber proportions compared with age-matched normal rats, as already mentioned. Specifically, the dominant fast fatigue intermediate type-IID/X myofiber proportion increases slightly with long-term injury in spastic muscles (~10% increase), and there is a small loss of slow nonfatigable type-I myofibers (Harris et al. 2005). However, from a functional perspective, these changes are small in relation to the initial large changes in myofiber types before the development of spasticity, where type I and type IIA myofiber proportions are halved and type IID/X proportions are increased accordingly. Furthermore, these changes are very small in relation to the large losses of slow and fatigue resistant myofibers and the proportional increases in faster, more fatigable myofibers seen after SCI that is not associated with spasticity (Burnham et al. 1997; Lieber et al. 1986a; Talmadge et al. 1995). Thus the relatively small changes in myofiber types seen in spastic tail muscles are consistent with the possibility that the spastic activity preserves the myofiber type distribution.

Factors that influence fatigue after injury

The large increase in spastic tail muscle fatigability observed in this study cannot be attributed to the small increase in fast fatigue intermediate type IID/X myofibers we have observed in these same muscles (Harris et al. 2005). Metabolic factors likely play a role, given the very large change in fatigability (>50% reduction in fatigue index). For example, poor blood circulation to the tail, as might be expected because of reduced autonomic tone associated with SCI (Johnson et al. 1998; Yu 1998), might ultimately lead to compromised ATP availability during our 100-s fatigue tests. Perhaps this also would account for the contractures that build up over the course of these tests. A number of other metabolic factors can influence muscle fatigability, including plasma membrane density of Na⁺, K⁺-ATPase (Fowles et al. 2002), creatine phosphate availability (Hartkopp et al. 2003), and concentration or activity of key oxidative enzymes such as succinate dehydrogenase and cytochrome C oxidase (Haller et al. 1991; Hambrecht et al. 1997); these factors may play a role in the fatigue of spastic muscles.

Factors that don't influence twitch duration and size after injury

The slowing/prolongation of the muscle twitch after injury cannot be accounted for by changes in myofiber types because the proportion of slow type I myofibers does not increase in spastic tail muscles after chronic SCI (Harris et al. 2005). Furthermore, the increased twitch size after chronic injury and long-term spasticity cannot be accounted for by changes in myofibers because there are only small changes in myofiber distributions, with an increase in the type IID/X myofibers of only 10%, which is countered by some atrophy in the type IID/X myofibers (Harris et al. 2005). Altered pennation angle can affect muscle force production, but this usually occurs in conjunction with myofiber hypertrophy (Aagaard et al. 2001); instead of hypertrophy, we saw a moderate atrophy of muscle in spastic chronic spinal rats compared with normal rats. Changes in muscle stiffness can also affect muscle force production; this force-stiffness interaction is dependent to a large extent on myofiber type composition (Malamud et al. 1996; Mirbagheri et al. 2001). However, we have found that stiffness does not change with injury in spastic tail muscles (unpublished observations) and, again, that myofiber type composition does not change much in spastic chronic spinal rats (Harris et al. 2005). Finally, changes caused by muscle maturation over the 8 mo of

these experiments are also unlikely to account for changes in contractile properties, because we saw no major changes in old age-matched normal rats compared with young normal rats, consistent with limited changes in contractile properties observed over this age range by Ansved and Larsson (1989).

Possible changes in intracellular calcium buffering and sequestering

One possibility that might account for many of the mechanical changes seen in spastic tail muscle is impaired myoplasmic calcium buffering (e.g., by parvalbumin) and/or decreased calcium sequestering into the sarcoplasmic reticulum (Jiang et al. 1996). Such changes would result in calcium staying in the myoplasm longer during a twitch (Schwaller et al. 1999). Thus force generation would be prolonged and the twitch force would reach a larger peak until calcium was sequestered into the sarcoplasmic reticulum. This scenario seems consistent with the prolonged and much larger twitches seen in spastic muscles. The concept that the decay of myoplasmic calcium is a major factor in determining twitch duration and amplitude has support in a number of other experimental systems where Ca^{2+} handling is rate limiting (Allen and Westerblad 1995; Choisy et al. 2001; Raymackers et al. 2000; Schwaller et al. 1999; Stein et al. 1982). In contrast, calcium decay should not affect the absolute maximum rate of rise of force during a twitch or a tetanus, so impaired calcium handling caused by SCI should not affect these parameters, consistent with our observations. Also, during maximal repetitive stimulation yielding a tetanus, there should be no lack of free calcium regardless of the calcium buffering/sequestering; thus the rise in force during a tetanus and the maximal tetanic force should not be affected by myoplasmic calcium handling, apparently consistent with our results. Certainly, the spastic tail muscle atrophied and produced less (not more) tetanic force, but it is compelling that the tetanic force normalized by the muscle mass is not different between normal and spastic muscles and that the rate of rise of tetanic force did not change. Ultimately, this should lead to a larger twitch-to-tetanus ratio, as also observed here. Furthermore, the decay of tetanic force is significantly slower in spastic muscles, again implying poor posttetanus calcium handling (Raymackers et al. 2000). Thus in general, with the exception of changes in fatigue, all changes in the muscle twitch and tetanus in spinal cord-injured rats with spasticity may be consistent with a compromised myoplasmic calcium buffering/sequestering system that occurs without major changes in contractile proteins (myosin heavy chain-identified myofiber proportions; Raymackers et al. 2000; Schwaller et al. 1999).

The spastic activity after SCI may produce a decrease in myoplasmic calcium buffering in the same way that electrical stimulation and exercise have been shown to decrease calcium buffering and sequestering in the hindlimb muscles of rats (Green et al. 1984; Huber and Pette 1996). Specifically, endurance training in rats induces a decrease in myoplasmic parvalbumin content (Green et al. 1984), a protein that normally serves to buffer calcium from troponin C until it is sequestered by the sarcoplasmic reticulum (Jiang et al. 1996). Training also markedly decreases the concentration of the sarcoplasmic reticulum (SR) Ca^{2+} -ATPase that pumps calcium into the sarcoplasmic reticulum during sequestration (Green et al. 1984). Additionally, when parvalbumin is eliminated in parvalbumin knockout mice, the twitch is of increased duration and increased peak amplitude (Schwaller et al. 1999) and relaxation from tetanus is slower (Raymackers et al. 2000), remarkably consistent

with our own observations in spastic muscles. The opposite effect is observed for twitch contractions after transgenic overexpression of parvalbumin in mice; in these mice, in contrast to our own findings, twitch contraction and relaxation are faster and tetani fuse and reach maximum tetanic force at higher frequencies than in normal mice (Chin et al. 2003).

Importantly, myosin heavy chain-identified myofiber types are not changed in parvalbumin-knockout mice (Schwaller et al. 1999) or in mice overexpressing parvalbumin (Chin et al. 2003), and changes in parvalbumin and SR Ca^{2+} -ATPase occurred well in advance of altered myosin ATPase-identified myofiber types over 15 wk of intensive exercise (Green et al. 1984). These data emphasize that calcium-handling proteins such as parvalbumin and the SR Ca^{2+} -ATPase can be altered independently of myofiber type transformations. Again, this appears consistent with our results in segmental tail muscles of chronic spinal rats, in which the twitch properties change as though calcium buffering might be compromised, with little change in myosin heavy chain-identified myofiber distribution.

Other investigators have found injury-induced or humoral changes in calcium handling with results similar to those seen in spastic tail muscles. For example, 7-day denervation causes reduced calcium uptake into the sarcoplasmic reticulum in rat fast fatigable type IIB myofibers and is associated with slowing of twitches and increased peak twitch (Germinario et al. 2002). Likewise, the opposite effects are seen in the soleus of hyper-thyroid rats in which calcium reuptake by the SR is increased (Fitts et al. 1980).

Summary

Overall, spastic tail muscles from chronic spinal rats are more fatigable than tail muscles from normal rats, likely because of changes in metabolic factors rather than changes in myosin heavy chain-identified myofiber types. The spastic muscles also have considerably slower contractile properties than normal muscles, including prolonged twitch contraction and relaxation, slower relaxation from tetanus, and lower fusion frequencies. Together with larger twitches, these results may be most consistent with altered intracellular calcium handling. Surprisingly, the slowing of active spastic muscle after long-term SCI is not associated with increased slow type I myofibers (Harris et al. 2005).

Importantly, slowing of muscle with SCI and spasticity is not unprecedented in humans. That is, human triceps surae and thenar muscles that are spastic after long-term SCI have considerably slower contraction speeds compared with those in uninjured humans (Hidler et al. 2002; Thomas 1997; Zijdwind and Thomas 2003). This effect is notably spasticity-dependent, with the most severe spasticity resulting in the most enhanced slow properties (Hidler et al. 2002). Thus spastic muscle activity is associated with preserved or even enhanced slow contractile properties.

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FIG. 1.

Twitch properties of the 14th ventrolateral segmental tail muscle of old normal rats and of rats with spasticity after chronic sacral spinal cord injury in response to supramaximal stimulation pulses. Representative twitches from normal (—) and chronic spinal (—) animals show that peak twitch force is much greater in chronic spinal animals (A). When these twitches are normalized to peak twitch force, it is clear that contraction and relaxation are both prolonged in spastic tail muscles from chronic spinal animals (B), and not just larger. Time to peak twitch (C) is the time required to achieve peak twitch force beginning at the onset of force production. Twitch half-rise time (D) is the time required to achieve one-

half of peak force (see 0.5 on vertical scale of *B*) beginning at onset of force production. Twitch force (*E*) is taken as the absolute maximal twitch force, and force:mass ratio (*F*) is the ratio of twitch force to muscle mass. In this and subsequent figures, white bars are normal animals and black bars are chronic spinal. *Significant difference from old normal rats and †significant difference from young normal rats in this and subsequent figures ($P < 0.05$). Values shown are means $\pm$ SD.

FIG. 2.

Representative traces from both old normal (*A*) and chronic spinal (*B*) rats show that twitch contractions of segmental tail muscles can be separated into twitches of their constituent motor units. With nerve stimulation of slowly increasing intensity, discrete increases in twitch force were seen (*a–g* in *A* and *a–h* in *B*), representing successive recruitment of individual motor units. Means of successive motor unit twitches that make up the muscle twitch were subtracted as indicated to reveal constituent motor units. For example, $c - b = 1$ in *A* and $d - c = 1$ in *B*. Motor units are arranged from fastest (1) to slowest (6 or 7) twitch contraction time. Note that slowest units in the chronic spinals reached their peak force ~50

ms after force onset, whereas faster units completed their twitch by this time. Thus twitch force at 50 ms (TT_{50}), normalized to peak twitch force, shows the overall “slowness” of a tail muscle (*C*). Bar graph format as in Fig. 1. Significance accepted at $P < 0.05$.

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FIG. 3.

Maximum rates of twitch rise and twitch decay determined from the derivative of twitch. Absolute max rise rate (*A*) is maximum rate of rise of twitch force development in the twitch, and absolute max decay rate (*B*) is maximum rate of muscle relaxation after peak twitch force. Normalized rise (*C*) and decay (*D*) rates are rates of rise and decay of twitch normalized to peak force. Bar graph format as in Fig. 1. Significance accepted at $P < 0.05$.

FIG. 4.

Representative tetani and their relationships to twitch contractions are shown for old normal rats (A) and chronic spinal rats (B). Tetani were elicited with supramaximal stimulation at 200 Hz for 500 ms. Twitches were produced by single, supramaximal stimulation pulses delivered 1,200 ms before and 1,200 ms after the onset of tetanus. In A and B, twitch before and twitch after are shown on an enlarged scale to the *right* of the full-scale traces. Tetanic force (C) is the peak force achieved during a single tetanus, and normalized tetanus (D) is the ratio of tetanic force to muscle mass. Note that tetanic force production is significantly smaller in tail muscles from chronic spinal rats (C). However, when the ratio of tetanic force to muscle mass is calculated, decrease in tetanic force with spinal cord injury seems to be accounted for by muscle mass atrophy (D). Tetanus half-rise time (E) is time required to reach one-half of peak tetanic force. Tetanus half-fall time (F) is time required after the end of tetanic stimulation for muscle force to be reduced by half. Posttetanic potentiation (G) is the ratio of peak twitch force after a tetanus to peak twitch force before a tetanus. Twitch:tetanus ratio (H) is the ratio of peak force of the nonpotentiated twitch (twitch before) to peak force of the tetanus. Bar graph format as in Fig. 1. Significance accepted at $P < 0.05$.

FIG. 5.

Fusion properties of segmental tail muscles. *A*: forces developed during supramaximal tetanic stimuli (300 ms) at frequencies from 10 to 200 Hz are shown for chronic spinal rats (●, error bars upward), old normal rats (○, error bars downward), and young normal rats (□, error bars upward). Note that symbols for old normal rats largely overlay those for young normal rats because values for these groups are nearly identical. Values are normalized to maximum tetanic force at 200 Hz. *B*: F_{50} is the frequency required to reach half-maximal tetanic force (0.50 on vertical scale in *A*). Force frequency curve for each animal was fit to a sigmoidal function ($r^2 = 0.9$ for all sigmoidal fits), and F_{50} was calculated

for each animal using its sigmoidal function. In chronic spinal rats relative to normal rats, F_{50} is achieved at a significantly lower stimulation frequency. Bar graph format as in Fig. 1. Significance accepted at $P < 0.05$.

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FIG. 6.

Fatigue with repeated contractions. Muscles were fatigued by producing 300-ms tetanic contractions (each with supramaximal stimulation at 40 Hz), repeated once per second for 100 s. Representative traces show the 1st and 100th tetani of the test for segmental tail muscles in old normal rats (*A*) and chronic spinal rats (*B*). *Insets*: ongoing change in peak tetanic force over 100 sweeps. Note that peak force in repeated 40-Hz trains potentiates slightly before declining, as shown in *insets* of *A* and *B*, and declines more in chronic spinal rats. Note also that force scales in *A* and *B* differ. To quantify fatigability, fatigue index (*C*) was calculated as the ratio of tetanic force produced in the 100th contraction to tetanic force

produced in the 1st contraction. Both forces were measured relative to baseline force before the 1st contraction. Bar graph format as in Fig. 1. Significance accepted at $P < 0.05$.

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Experimental (chronic spinal rats) and control (young and old normal rats) animals used in assessing segmental tail muscles in chronic spinal cord-injured rats

Table 1

Group	n	Age, mo	Mass, g	Wet Tail Muscle Mass, mg	Tail Muscle-to-Body Mass Ratio, mg/kg
Young normal rats	8	3.0 ± 0.6	299 ± 21	13.7 ± 2.4	45.6 ± 8.5
Old normal rats	5	9.7 ± 1.1 [†]	386 ± 36 [†]	17.6 ± 2.3 [†]	45.9 ± 11.5
Chronic spinal rats	5	10.0 ± 1.7 [†]	415 ± 74 [†]	13.4 ± 1.9 [*]	33.2 ± 7.7 ^{*†}

Values are means ± SD.

^{*} Significant difference from old normal rats and

[†] significant difference from young normal rats ($P < 0.05$).