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Forelimb Muscle Plasticity Following Unilateral Cervical Spinal Cord Injury

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Abstract

Introduction—Motor dysfunction and muscle atrophy are well-documented in the lower extremity following spinal cord injury. However the extent and time course of myoplastic changes in forelimb musculature is not clear.

Methods—Forelimb muscle morphology and fiber type were evaluated following high cervical hemi-lesion injury in rats.

Results—There was significant atrophy of the ipsilateral extensor carpi radialis longus (ECRL) muscle 2 weeks post-injury, which was subsequently reversed at 8 weeks post-injury. The triceps muscle showed minimal evidence of atrophy following spinal injury. No significant changes in fiber type were observed.

Discussion—These findings indicate a robust capacity for spontaneous myoplasticity following C2 hemisection injury but highlight differential capacity for plasticity within the forelimb muscles.

Keywords

Spinal cord injury; upper extremity; myoplasticity; atrophy; forelimb muscle; spontaneous recovery

Introduction

Myofiber atrophy and impaired muscle function contribute to motor deficits and are well documented in the lower extremity^{1,2} and other motor systems³ following spinal cord injury (SCI). While upper extremity muscles are directly (e.g., denervation) or indirectly (e.g.,

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compensatory changes, inflammation) affected by cervical SCI, the time course and extent of myoplastic forelimb changes has not been evaluated formally. We addressed this knowledge gap by examining forelimb muscle morphology after unilateral cervical SCI. A rodent C2 hemi-lesion (C2Hx)⁴ model was used to remove ipsilateral descending synaptic inputs to forelimb motoneuron pools and thus transiently paralyze the ipsilateral forelimb. The extensor carpi radialis longus (ECRL) and triceps brachii (TRI) muscles were evaluated, as they are functionally important for locomotion, weight-bearing, grooming, and feeding.

Materials and Methods

All procedures were approved by the University of Florida Institutional Animal Care and Use Committee. The following groups of female Sprague-Dawley rats (248 ± 2 g; Harlan Inc; Indianapolis) were studied: uninjured control ($n=6$), 1 week ($n=8$), 2 week ($n=8$), and 8 weeks post-C2Hx ($n=8$). Anesthesia, SCI surgery, and animal care were performed as described previously⁵⁻⁷ (also see online supplemental methods). At the predefined endpoint, rats were anesthetized with xylazine (10mg/kg, s.q.) and ketamine (140 mg/kg, i.p., Fort Dodge Animal Health, IA, USA), and the TRI and ECRL were dissected and processed as previously described.^{3,8} Following muscle retrieval, rats were euthanized by systemic perfusion with saline followed by 4% paraformaldehyde (Sigma, St. Louis, MO, USA). Spinal lesions were examined according to previously published methods,^{4,9} as described in the supplemental online section; 2 animals with histologically incomplete hemi-lesions were excluded.^{4,9} Muscle wet weights were assessed and muscle fiber cross sectional area (CSA, μm^2) were calculated using Leica Application Suite software (version 3.5.0). Average CSA was determined based on analysis of 250-350 myofibers.^{3,8} A paired *t*-test confirmed the absence of laterality differences in uninjured controls for both wet weight ($P > 0.3$) and average muscle fiber CSA ($P > 0.536$ for all fiber types), thus left and right data points were pooled for both TRI and ECRL muscles. Overall statistical comparisons of muscle wet weight and myofiber size across control and C2Hx groups were made using 1-way analysis of variance (ANOVA). The Holm-Sidak multiple comparison test was used to compare data at 1, 2, and 8 wks post-C2Hx to control values. All data are expressed as the mean $\pm$ 1 standard error or as a % change from the non-injured control values; significance was set at $P < 0.05$.

Results

The wet weight of the ECRL was different across control and injured rats ($P < 0.001$). Specifically, weight was reduced by 17% ($P < 0.001$ vs. control) and 14% ($P < 0.001$ vs. control) on the ipsilateral and contralateral sides, respectively, at 1 wk post-injury, and returned to control uninjured values by 2 wks (Figure 1-A, top panel). The mean CSA of ipsilateral ECRL type I fibers was also different across experimental groups ($P = 0.003$), with a reduction at 2 wks post-injury ($P = 0.003$ vs. control), followed by a slight recovery by 8 wks (Figure 1-B). Type IIa ($P = 0.011$) and IIb/x ($P = 0.014$) ipsilateral ECRL myofiber CSA were also different across groups. In both cases, the Holm-Sidak test indicated reduced size at 2 wks post-C2Hx (IIa, $P = 0.009$; IIb, $P = 0.025$). No changes in mean myofiber CSA of contralateral ECRL were observed in any fiber type at any post-injury time point (ANOVA: $P > 0.485$ for all).

TRI muscle wet weight was altered by C2Hx ($P < 0.001$) with reductions of 18% ($P < 0.001$) and 10% ($P < 0.001$) on the ipsilateral and contralateral sides, respectively, after 1 wk. The reduction in weight was transient with values returning to control by 2 wks (Figure 1-A, bottom panel). The 1-way ANOVA did not reveal any significant differences in TRI myofiber CSA across experimental groups (all $P > 0.372$; Figure 1-C), although some trends can be observed in the data. Specifically, modest reductions in ipsilateral fiber size were observed in type IIb/x and type IIa muscle fibers at 1 and 2 weeks post-injury respectively. Both the ECRL and TRI muscles are comprised primarily of type II muscle fibers (48 and 36% type IIa and 40 and 50% type IIb/x respectively), and these proportions did not change at any post-injury time point (ANOVA: $P > 0.4$ for all; data not shown).

Discussion

The C2Hx lesion is characterized by complete loss of descending ipsilateral inputs to caudal motoneurons but is likely to produce minimal, if any, motoneuron loss^{10,11} below the level of injury^{10,11}. Thus, with respect to forelimb motor function, interruption of white matter tracts is likely to be the most significant impact of C2Hx, although propriospinal gray matter tracts may also be functionally important¹². The myoplastic response of the muscles innervated by motor pools caudal to the level of injury will be influenced by a complex interplay between diminished muscle activity (secondary to motoneuron denervation), and subsequent spinal plasticity leading to activation of spared motoneurons.^{4,9,11,13,14} We observed a transient reduction and subsequent recovery of ipsilateral ECRL muscle fiber size after C2Hx, and this pattern generally corresponds to the functional recovery of the ipsilateral forelimb following this injury.¹⁰ Taken together, these observations are suggestive of time-dependent activation of “crossed-spinal” synaptic pathways to the ipsilateral ECRL motor pools^{10,15}. In other words, restoration of function is likely to be at least partly mediated by descending mono- and poly-synaptic projections that cross the spinal midline caudal to the lesion site to innervate ipsilateral motoneurons similar to the “spontaneous crossed phrenic phenomenon”, which occurs in the phrenic motor system.^{4,9,11,13,14} Polysynaptic spinal pathways are likely to be involved, since ECRL motoneurons receive synaptic inputs from a complex propriospinal network,¹⁰ and spinal interneurons represent an integral part of the neural substrate underlying motor recovery in other examples of SCI.^{16,17} Non-neural mechanisms related to systemic inflammation may also have influenced the myoplastic responses observed here. Indeed, SCI induces a cascade of inflammatory responses, including systemic upregulation of circulating cytokines, and this can trigger skeletal muscle atrophy and contractile dysfunction.¹⁸ However, the divergent responses between ipsilateral and contralateral muscles suggest that the initial ipsilateral ECRL atrophy resulted, at least in large part, from reduced neural activity within the ipsilateral forelimb motoneuron pools secondary to the C2Hx injury. Future experiments designed to tease out the specific mechanisms (i.e. neural versus non-neural) underlying the observed myoplastic responses will be necessary to fully understand the impact of reduced neural activity on muscle fiber characteristics.

The lack of atrophy observed in the TRI muscle represents an interesting divergence from the pattern of atrophy observed in the ECRL muscle. One possibility is that crossed spinal pathways innervating the TRI muscle are more prevalent, and thus the ability to activate it is

preserved and the functional impact of the C2Hx injury is minimized. Another consideration is that the ECRL is integrally involved in skilled motor behaviors such as reaching and grasping, and ECRL motoneuron activation may therefore have greater reliance on ipsilaterally descending corticospinal inputs as compared to the triceps muscle.¹⁹ Lastly, the divergent myoplastic responses observed between the ECRL and TRI muscles may simply be the result of inherent differences between these skeletal muscles (e.g., differences in fiber type composition or muscle innervation) that were not specifically assessed in this study.

In conclusion, these data demonstrate muscle-specific forelimb responses to unilateral high cervical SCI with transient atrophy of the ECRL. The C2Hx model thus provides a platform for subsequent investigations of the mechanisms underlying forelimb myoplasticity after SCI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ECRL	Extensor Carpi Radialis Longus
SCI	Spinal cord injury
C2Hx	C2 hemi-lesion
CSA	Cross sectional area
TRI	Triceps Brachii
s.q.	sub-cutaneous
i.v.	intravenous

Figure 1.

Muscle wet weight and fiber size for extensor carpi radialis longus (ECRL) and triceps (TRI) muscles in spinal intact vs. C2Hx rats. (A) Muscle wet weights of ECRL and TRI muscles of spinal intact, 1-, 2-, and 8-week C2Hx rats. (B) Mean cross sectional area of Type I, IIa, and IIb/x myofibers from ipsilateral and contralateral ECRL muscles of spinal intact, 1-, 2-, and 8-week C2Hx rats. (C) Mean cross sectional area of Type I, IIa, and IIb/x myofibers from ipsilateral and contralateral TRI muscles of spinal intact, 1-, 2-, and 8-week C2Hx rats. (D) Representative cross sections taken from the ipsilateral ECRL muscles of spinal intact, 1-, 2-, and 8-week C2Hx rats. Sections were incubated with an anti-dystrophin antibody (red) for visualization of myofiber membrane and anti-MHC type I (blue) and anti-MHC type IIa (green) antibodies; non-labeled profiles are presumed to be type IIb/x fibers. Scale Bar = 200µm; Bars represent mean ± SEM. *Holm-Sidak: $P < 0.05$ vs. control.