Administration of Chondroitinase ABC Rostral or Caudal to a Spinal Cord Injury Site Promotes Anatomical but Not Functional Plasticity

Veronica J. Tom, Rachel Kadakia, Lauren Santi, and John D. Houle

Abstract

Growth-inhibitory chondroitin sulfate proteoglycans (CSPG) are a primary target for therapeutic strategies after spinal cord injury because of their contribution to the inhibitory nature of glial scar tissue, a major barrier to successful axonal regeneration. Chondroitinase ABC (ChABC) digestion of CSPGs promotes axonal regeneration beyond a lesion site with subsequent functional improvement. ChABC also has been shown to promote sprouting of spared fibers but it is not clear if functional recovery results from such plasticity. Here we sought to better understand the roles rostral or caudal sprouting may play in ChABC-mediated functional improvement. To achieve this, ChABC or vehicle was injected rostral or caudal to a unilateral C5 injury. When injected rostral to a hemisection, ChABC promoted significant sprouting of 5HT+ fibers into dorsal and ventral horns. When ChABC was injected into tissue caudal to a hemisection, no additional sprouting was observed. When injected caudal to a hemicontusion injury, ChABC promoted sprouting of 5HT+ fibers into the ventral horn but not the dorsal horn. None of this sprouting resulted in a change in the synaptic component synapsin, nor did it impact performance in behavioral tests assessing motor function. These data suggest that ChABC-mediated sprouting of spared fibers does not necessarily translate into functional recovery.

Key words: chondroitinase ABC; plasticity; serotonergic fibers; spinal cord injury; sprouting

Introduction

IN THE DEVELOPING CENTRAL NERVOUS SYSTEM (CNS), a family of potent axon growth-inhibitory extracellular matrix molecules called chondroitin sulfate proteoglycans (CSPG) are important for proper axonal guidance and boundary formation during development (Brittis et al., 1992; Snow et al., 1990). CSPGs are comprised of a protein core to which large glycosaminoglycans (GAGs) are covalently attached. These GAGs are thought to confer most of CSPG's axonal growth-inhibitory properties. When CSPG-containing substrates are treated with the bacterial enzyme chondroitinase ABC (ChABC) to cleave the GAGs from the protein core, the substrates become more growth permissive (Snow et al., 1990), supporting the notion that the GAGs bestow inhibitory characteristics upon CSPG.

While CSPG expression is largely diminished in the adult CNS, they are present in the perineuronal nets that encapsulate mature neurons and are thought to maintain synaptic contacts and limit plasticity, presumably because of the inhibitory nature of CSPG (Galtrey and Fawcett, 2007). Indeed, ChABC-mediated digestion of perineuronal nets reopens the

developmental critical period and promotes functional plasticity in both intact and injured mature nervous systems (Massey et al., 2006; Pizzorusso et al., 2002).

CSPGs are upregulated in the adult CNS following injury. Increased expression levels of multiple members of CSPG within the lesion penumbra (Jones et al., 2003; Tang et al., 2003) is thought to be a major cause of injured adult axons failing to successfully regenerate past the injury site (Fawcett, 2006; Silver and Miller, 2004). Thus, ChABC has been used to digest scar-associated CSPGs following injury, resulting in increased axonal regeneration and some functional recovery (Bradbury et al., 2002; Caggiano et al., 2005; Fouad et al., 2005; Houle et al., 2006; Tester and Howland, 2008). However, there has been only one published report in which promoting true axonal regeneration has been directly linked to behavioral recovery (Houle et al., 2006). In that study, the severing of a peripheral nerve graft bridge filled with regenerated axons eliminated functional improvement that was seen following ChABC treatment of the injury site.

One issue clouding the mechanism behind ChABC-mediated recovery of function is that ChABC has been demonstrated to also promote axonal sprouting of fibers in both

intact (Barritt et al., 2006; Corveti and Rossi, 2005) and injured systems (Barritt et al., 2006). In particular, intrathecal delivery of ChABC following a cervical dorsal column lesion promotes the sprouting of intact serotonergic fiber populations, which may be responsible for some of the recovery seen in the previous studies. To further understand the role that sprouting, non-regenerating fibers play in functional recovery following ChABC treatment, we microinjected ChABC (Tom and Houle, 2008) exclusively into tissue rostral or caudal to a hemisection injury and caudal to a hemicontusion injury to test directly whether sprouting of spared fibers rostral or caudal to the injury was promoted and if either was sufficient to augment recovery.

Methods

Surgical procedures

All procedures complied with Institutional Animal Care and Use Committee and NIH guidelines for experimentation with lab animals. Two injury models, a cervical hemisection and a cervical hemicontusion, were used. For hemisection injuries, adult female Sprague-Dawley rats (225–250 g, Charles River, Wilmington, MA) were anesthetized with isoflurane. A partial laminectomy was performed on the fifth cervical vertebra (C5) to expose the right half of the spinal cord, and the dura was cut to expose the dorsal spinal cord. The entire right half of the C5 spinal cord was removed by aspiration, resulting in a cavity that was 2–3 mm in length. The dura was closed using a 10-0 suture. To determine the effect of ChABC on sprouting rostral to the injury, animals that received C5 hemisections underwent partial laminectomy of C3 and C4 vertebral processes. Either PBS ($n=7$) or ChABC ($n=8$; 50 U/mL; Seikagaku) was microinjected unilaterally throughout C3–C4 using pulled glass pipettes (0.5 μ L per site, 4–5 injection sites in a straight line in the middle of the exposed right side of the spinal cord). To determine the effect of ChABC on sprouting caudal to a C5 hemisection injury, partial laminectomies were performed on C6 through C8 vertebrae to expose the dorsal surface of ipsilateral spinal cord caudal to the injury site. Either PBS ($n=8$) or ChABC ($n=8$) was microinjected throughout C6–C8 (0.5 μ L per site, 8–9 injection sites per animal). A piece of silastic membrane was placed over the length of the sutured dura. The overlying musculature was closed using 4-0 sutures and the skin was closed using wound clips.

For hemicontusion injuries, adult female Sprague-Dawley rats were injured as described previously (Sandrow et al., 2008). Briefly, animals were injected intraperitoneally with ketamine (60 mg/kg) and xylazine (10 mg/kg). The right dorsal surface of C5 was exposed by laminectomy. The vertebral column was stabilized by clamping the C3 and C7 vertebral bodies with forceps fixed to the base of an Infinite Horizon Impact Device (Precision Systems and Instrumentation, Lexington, KY). The animals were situated on the impactor and the 1.6 mm stainless steel impactor tip was positioned over the midpoint (medial to lateral) of the right side of C5. The animals were impacted with a 200 kdyn force with displacement of tissue to a depth of 1600–1800 μ m. Clamps were released and partial laminectomies were performed on C6–C8 to expose the dorsal surface of ipsilateral spinal cord caudal to the hemicontusion. Either PBS ($n=7$) or ChABC ($n=8$; 50 U/mL) was microinjected as described

above. Silastic membrane was placed over the sutured dura, the overlying musculature was sutured shut, and the skin was closed with wound clips.

All animals were given ampicillin (200 mg/kg) and buprenorphine (0.1 mg/kg) postoperatively and placed on a thermal barrier to recover. They were returned to their cages once they became alert and responsive.

Six weeks after injury, animals were given an overdose of Euthasol (Sigma, St. Louis, MO) and were transcardially perfused with ice-cold 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB; pH 7.4). The spinal cords were dissected out, post-fixed in 4% PFA overnight at 4°C, and then transferred to 30% sucrose in PB (pH 7.4).

Behavioral testing

Rats were allowed to acclimate to their new environment for 1 week before baseline abilities were scored. Rats were tested 2–3 days after spinal cord injury and weekly thereafter for at least 6 weeks. The grid walking and forelimb locomotor scale (FLS) tests were videotaped. All tests were scored by trained observers who were blinded to the experimental treatment.

Grid-walking test

This test was administered as described previously (Shumsky et al., 2003). Briefly, the animals were placed on a plastic-coated wire mesh grid for 2 min, and the number of forepaw placements on the bars was counted. A correct placement was defined as a step in which the paw gripped the bar and supported body weight. The number of correct placements was expressed as a percentage of the total steps. Scores of PBS- and ChABC-treated animals at each time point were compared for significance using a one-way ANOVA followed by a two-tailed Student's *t* test (SPSS, Chicago, IL).

FLS test

The BBB open-field locomotor test that was developed to evaluate hindlimb function after midthoracic contusion injuries (Basso et al., 1995) was used as a model to assess the affected right forelimb since these experiments involved unilateral cervical lesions. The FLS was devised to reflect the typical pattern of deficit and recovery and qualitatively examines parameters of locomotion, including use of forelimb joints, extent of weight support, plantar placement of the affected forepaw, and forepaw rotation. Rats were placed in an enclosure (1.5×0.6 meters) and activity was videotaped for 4 min. The FLS scores range from 0 (no function) to 17 (no deficit) (Cao et al., 2008; Sandrow et al., 2008). Scores of PBS- and ChABC-treated animals were analyzed for significance using one-way ANOVA and post hoc Mann-Whitney tests for each time point.

Histology

Transverse sections were cut on a cryostat at 30 μ m and collected in a series of six in PBS. Sections were rinsed in PBS, blocked for 1 h at room temperature with 5% normal goat serum, 1% bovine serum albumin, 0.1% Triton X-100 in PBS, and then incubated in the appropriate primary antibody overnight at 4°C. The primary antibodies used were rabbit

anti-5HT (ImmunoStar, Hudson, WI), rabbit anti-synapsin (Chemicon, Temecula, CA), and mouse anti-chondroitin sulfate "stub" (2B6, Seikagaku, Tokyo, Japan; an antibody that recognizes the four-sugar stub that remains following ChABC digestion of CSPG). Some sections were also incubated with *Wisteria floribunda* agglutinin (WFA; Sigma, St. Louis, MO) to label intact perineuronal nets (Bruckner et al., 1998). The sections were rinsed in PBS and incubated in the appropriate preabsorbed secondary antibody overnight at 4°C. The sections were rinsed, mounted on glass slides, coverslipped using VectaShield (Vector Laboratories, Burlingame, CA), and sealed with nail polish. All sections were viewed using a Zeiss Axioskop microscope, and images were taken with a CoolSnap CCD digital camera and a Leica TCS SP2 confocal 20× lens.

Quantification of histology

To quantify all immunohistochemical reactions, four sections within C4 (for rostral studies) or C6 (for caudal studies) from PBS- or ChABC-treated animals 6 weeks after injury were selected. For 5HT-stained sections, fluorescent images of dorsal and ventral horns ipsilateral to the injury were taken using identical exposure times. For synapsin-stained sections, images were taken of ipsilateral ventral horn. All images were thresholded to eliminate background and account only for 5HT+ profiles (MetaMorph, Universal Imaging Corp., Downingtown, PA). The same threshold value was used to analyze all images. The threshold pixel area within a fixed-size region in the area of interest was measured and compared for statistical significance between PBS- and ChABC-treated animals using a Student's *t* test (Microsoft Excel, Tacoma, WA).

Results

Microinjection of ChABC into intact tissue adjacent to a spinal cord injury site digests perineuronal nets

Previous studies have determined that treatment with ChABC digestion of CSPG-rich perineuronal nets surrounding neurons allows for synaptic plasticity (Bruckner et al., 1998; Massey et al., 2006; Pizzorusso et al., 2002). To ensure that our microinjection technique to deliver ChABC (Tom and Houle, 2008) was sufficient to digest perineuronal nets, acutely injured C5 hemisectioned animals were injected with ChABC or PBS at C6 and sacrificed 10 days later. We initially used ChABC at a concentration of 20 U/mL but found that that did not completely digest perineuronal nets (data not shown). Therefore, we switched to a higher concentration that was successfully used to digest nets within the brainstem (Massey et al., 2006). WFA was used to identify intact perineuronal nets, and the CSPG stub antibody 2B6 was used to identify digested perineuronal nets in C6 spinal cord sections. In PBS-treated animals, motoneurons within the ventral horn caudal to the injury site were surrounded by intense WFA staining (Fig. 1A, arrows), indicating that perineuronal nets encapsulating these neurons were intact. There was sparse 2B6 staining (Fig. 1B) in these animals. Ten days following ChABC treatment, WFA staining around motoneurons was abolished (Fig. 1C, arrowheads) but there was strong 2B6 immunoreactivity (Fig. 1D, arrowheads), indicating that microinjecting ChABC effectively digested CSPGs within perineuronal nets.

ChABC treatment rostral or caudal to a hemisection promotes sprouting of serotonergic fibers

Digestion of CSPGs by ChABC within perineuronal nets may serve two functions: reopening the window for synaptic plasticity and removing some CSPG-mediated inhibition of outgrowth to promote axonal sprouting of fibers. Thus, we next determined if ChABC administration rostral or caudal to a C5 hemisection was sufficient to promote sprouting of 5HT fibers into spinal cord tissue ipsilateral to the injury. Six weeks following a C5 hemisection and *rostral* microinjection of ChABC into C3–C4, there was significantly more 5HT immunoreactivity ipsilateral to the lesion at C4 in the dorsal and ventral horns of ChABC-treated animals (Fig. 2B and D, respectively) than in vehicle-treated animals (Fig. 2A and C). There was approximately twice as much 5HT staining in rostral dorsal horn (Fig. 2B; $p < 0.001$) and rostral ventral horn (Fig. 2D and E; $p < 0.001$) following ChABC treatment.

Six weeks after ChABC injection into tissue *caudal* to a C5 hemisection, there was no difference between the density of 5HT+ fibers within caudal ipsilateral ventral horns of ChABC- and PBS-treated animals (Fig. 2F). Although there was a trend for an increase in 5HT+ fibers in the dorsal horn after ChABC, it was not significant (Fig. 2F; $p < 0.15$).

Caudal administration of ChABC following a unilateral C5 contusion promotes serotonergic fiber plasticity

Since the hemisection lesions completely interrupted all fibers ipsilateral to the lesion, we wanted to determine if we would detect different results if ChABC were injected caudal to a more clinically relevant hemicontusion injury where many spared fibers remain ipsilateral and caudal to the lesion.

As with the hemisection-injured animals, PBS or ChABC was microinjected into C6–C8 immediately following a C5 unilateral contusion. At 6 weeks following injury, there was no significant difference in the 5HT immunoreactivity in the ipsilateral caudal dorsal horn between PBS- (Fig. 3A) and ChABC-treated animals (Fig. 3B and E). However, there was approximately 50% more 5HT immunoreactivity in the ventral horns of ChABC-treated animals (Fig. 3D) than that seen in the PBS-treated animals (Fig. 3C and E; $p < 0.01$). Thus, following a unilateral contusion, ChABC treatment of tissue caudal to the injury site promotes long-lasting plasticity of serotonergic fibers.

ChABC rostral or caudal to hemisection and hemicontusion injuries is not sufficient to enhance inherent functional recovery

Because ChABC disassembles plasticity-limiting perineuronal nets, we tested whether ChABC-induced sprouting of spared fibers rostral or caudal to an injury resulted in improved functional activity. To test this, PBS or ChABC was injected into multiple sites within C3–C4 or C6–C8 immediately following C5 hemisection or C5 hemicontusion. The injury site was not treated. Animals were tested weekly with the FLS to assess their abilities to use their right (affected) forelimb to locomote in the open field (Cao et al., 2008). Two to three days following a C5 hemicontusion, both PBS- and ChABC-treated animals had an average score of 3, indicating slight movement of all three joints of the affected forelimb. Within 2 weeks all animals had spontaneously recovered

FIG. 1. Microinjection of ChABC adjacent to a spinal cord injury digests perineuronal nets. (**A** and **B**) C6 spinal cord from a PBS-treated C5 hemisected animal 10 days following injury and injection that was double-stained for WFA and the CSPG “stub” antibody. PBS treatment had virtually no effect on perineuronal nets within the ventral horn, which were positively stained with WFA (**A**, arrows). There was no corresponding 2B6 staining in these animals (**B**). (**C** and **D**) C6 spinal cord section from an animal that was injected with ChABC. ChABC treatment abolished WFA-staining around motoneurons (**C**, arrowheads). These same neurons were surrounded by intense 2B6 immunoreactivity (**D**). Scale bar, 90 μ m.

significantly greater ability to use their affected forelimb ($p < 0.0001$). This recovery reached maximum by the third week when animals in both treatment groups had achieved average scores of 10 to 11, meaning they were frequently or continuously plantar stepping with their affected forelimbs (Fig. 4A). There was no difference between the PBS- and ChABC-treated groups at any of the tested time points, indicating that ChABC did not enhance the natural recovery of the injured animal to use its affected forelimb in the open field nor did it expand on the capabilities of forelimb movement once reaching a plateau. ChABC treatment rostral or caudal to the hemisection did not improve FLS scores compared to PBS treatment (data not shown).

There was a similar lack of effect of ChABC on promoting additional recovery in the grid-walking test. Several days following hemiconotusions and injection, PBS-treated animals had correct paw placements on the bars of the platform $2.6 \pm 2.6\%$ of the time, while ChABC-treated animals had

correct paw placements $7.4 \pm 5.1\%$ of the time (Fig. 4B). By the second week following injury, both groups of animals had significantly and maximally recovered some of their ability to correctly place their paw on the bars (PBS, $71.0 \pm 4.9\%$; ChABC, $80.0 \pm 3.7\%$). There was no difference between PBS or ChABC treatment at any time point, suggesting that treatment with ChABC did not enhance the spontaneous recovery. Neither rostral nor caudal ChABC enhanced grid walking in the hemisected animals (data not shown).

ChABC treatment does not affect synapse density

Despite the increased 5HT+ fiber sprouting, particularly within the ventral horn of ChABC-treated animals, there was no additional functional recovery. To determine if there is a difference in the density of a synaptic protein present due to PBS or ChABC treatment, C6 sections from PBS- or ChABC-treated animals sacrificed 6 weeks following a unilateral

FIG. 2. ChABC treatment rostral or caudal to a hemisection promotes plasticity of serotonergic fibers. (A–D) C4 spinal cord from a PBS-treated (A and C) or ChABC-treated (B and D) animal that was immunostained for 5HT 6 weeks after C5 hemisection. Following ChABC injection, there were significantly more 5HT+ fibers present in the dorsal horn (B) and in the ventral horn (D) compared to PBS injection. (E) Quantification of rostral 5HT+ immunostaining at 6 weeks following injury and injection. There were significantly more 5HT+ fibers within the dorsal and ventral horns of ChABC-treated animals than those of PBS-treated animals at 6 weeks after injury ($*p < 0.001$). (F) Quantification of 5HT+ immunostaining in C6 sections at 6 weeks following C5 hemisection and injection of PBS or ChABC into C6–C8. There were no significant differences in the density of 5HT+ fibers within the dorsal and ventral horns of ChABC-treated animals compared to that of PBS-treated animals. Scale bar, 60 μ m (A and B); 90 μ m (C and D).

FIG. 3. Caudal administration of ChABC following a unilateral C5 contusion promotes serotonergic fiber plasticity. (A–D) Representative images of C6 sections stained for 5HT from a PBS-treated (A and C) and ChABC-treated (B and D) hemi-contused animal 6 weeks after the injury. Similar amounts of 5HT immunoreactivity were present in the dorsal horns of PBS-treated (A) and ChABC-treated (B) animals. However, there was more 5HT+ staining within the caudal ventral horns of ChABC-treated animals (D) than of PBS-treated animals (C and E, $p < 0.01$). Scale bar, 60 μ m (A and B); 90 μ m (C and D).

FIG. 4. ChABC caudal to a hemicontusion injury is not sufficient to enhance inherent functional recovery. (**A** and **B**) Animals that were hemicontused at C5 and microinjected with PBS or ChABC throughout C6-C8 were assessed weekly for their abilities to locomote in the open field (**A**) and on a grid platform (**B**). While there was recovery over time following injury that plateaued by week 2–3 in both treatment groups, there was no enhancement of this spontaneous recovery with ChABC.

contusion were immunostained for synapsin to examine the overall density level of synaptic contacts within the ventral horn. PBS-treated animals had abundant punctate synapsin immunoreactivity throughout the ventral horn (Fig. 5A) and ChABC-treated animals had a similar staining pattern (Fig. 5B). Densitometric analysis revealed no significant difference in synapsin immunoreactivity following PBS or ChABC treatment within the ventral horn (Fig. 5C).

Discussion

We sought to determine if microinjection of ChABC into tissue rostral or caudal to a spinal cord injury site was suffi-

cient to promote sprouting of fibers, resulting in functional recovery. Because CSPG within the glial scar is a potent inhibitor of axonal regeneration following injury, many studies have used ChABC to digest this extracellular matrix component at the lesion site to attempt promotion of axonal regeneration. Most of these studies administered ChABC intrathecally, which diffusely distributes ChABC in the CNS (Garcia-Alias et al., 2007) such that tissue both rostral and caudal to the injury site are affected. In this study, ChABC was not administered to the injury site because we wanted to target sprouting and *not* regeneration across the lesion. Our results indicate that ChABC treatment of rostral tissue increases sprouting of 5HT+ fibers ipsilateral to the injury.

FIG. 5. ChABC treatment does not affect synapse number. (A and B) Representative images of ventral horn from sections of C6 from PBS-treated (A) and ChABC-treated (B) hemicontused animals that were stained for synapsin 6 weeks following injury. There was abundant punctate synapsin immunoreactivity within ventral horn ipsilateral and caudal to the hemi-contusion following both PBS- and ChABC-treatment. (C) Quantification of synapsin staining within the ventral horn. There was no difference in the degree of synapsin immunoreactivity between PBS- and ChABC-treated animals. Scale bar, 60 μ m.

Additionally, administering ChABC to tissue caudal to an injury effectively promoted sprouting of 5HT+ fibers.

We studied the effects of ChABC in two unilateral injury models: a hemisection and a hemicontusion. One of the main differences between the two injury types is the degree of spared tissue ipsilateral to the injury. Because the hemisection lesion interrupts all ipsilateral descending tracts, one can study the effects of ChABC in a more severe injury and maximize the likelihood of discerning plasticity and distinguishing a functional difference in the behavioral tests. Thus, we injected ChABC rostral only in the hemisection model because we hypothesized that ChABC may promote short-distance sprouting of injured fibers no longer connected to their target neurons onto propriospinal neurons, which are thought to be key mediators in functional plasticity (Bareyre et al., 2004; Courtine et al., 2008) rostral to the injury site. However, it is also possible that ipsilateral descending fibers spared from a hemicontusion injury or fibers contralateral to a

hemisection lesion would be able to sprout below the level of injury and reinnervate denervated ipsilateral target neurons and mediate recovery. Therefore, we also studied the effects of ChABC treatment caudal to a hemicontusion and a hemisection injury.

Treatment with ChABC caudal to a hemicontusion resulted in significantly more 5HT sprouting than when injected caudal to a hemisection injury (when comparing both treated groups to their respective controls). This likely is due to more fibers being spared from injury in the contusion model than in the hemisection model, increasing the numbers of fibers available to sprout after CSPG digestion. Additionally, the fact that a contusion injury produces a much larger inflammatory response than a laceration injury (Siegenthaler et al., 2007) may also contribute to the discrepancy. Because it involves both proinflammatory and anti-inflammatory cytokines, the heightened inflammatory response following a hemicontusion may have both detrimental and beneficial (i.e.,

growth-promoting) effects (Donnelly and Popovich, 2008; Gensel et al., 2009).

ChABC treatment after spinal cord injury has two potential benefits that may result in functional recovery. The first is that digestion of CSPGs within the glial scar may promote growth of injured and spared axons. The second is that ChABC will also digest CSPG within plasticity-limiting perineuronal nets, thus increasing the possibility of functional reorganization. Onifer and colleagues (Massey et al., 2006) injected ChABC into the dorsal column nuclei (DCN) to digest perineuronal nets following a dorsal column injury. The uninjured sensory fibers that entered the spinal cord above the level of the injury formed collateral sprouts in the DCN that were functionally compensatory. Interestingly, the window for any potential ChABC-enhanced plasticity appears to be fairly long, as digested perineuronal nets take several months to fully reconstitute themselves (Bruckner et al., 1998).

In the present study, however, there was no difference in functional recovery between ChABC- and PBS-treated groups despite the observed significant sprouting rostral and caudal to a cervical injury and the digestion of perineuronal nets in treated tissue. This is surprising because descending serotonergic inputs are involved in locomotion function (Jordan et al., 2008), and recovery in similar tests is often correlated with the presence of 5HT fibers caudal to a lesion (Kim et al., 2006). Moreover, it has been suggested that spontaneously occurring recovery of function seen in many spinal cord injury models is mediated not by regeneration of supraspinal pathways but by plasticity of propriospinal relay connections (Bareyre et al., 2004; Courtine et al., 2008). Because ChABC promotes sprouting of both injured and spared tracts (Barritt et al., 2006), one would have predicted that digestion of CSPG-rich perineuronal nets with ChABC would have enhanced the sprouting of supraspinal and propriospinal neurons and their ability to form synapses on neurons with "open" perineuronal nets, thus augmenting the natural recovery process. While we did not directly observe the effects of ChABC on propriospinal projections or a supraspinal population other than 5HT, we can extrapolate that even if other descending axonal tracts had sprouted caudal to the injury due to ChABC treatment, they did not establish functional connections, even though the perineuronal nets surrounding neurons within treated tissue had been disrupted. Our evidence for this is the finding that there was no significant difference in synapsin immunoreactivity within caudal ipsilateral ventral horn following vehicle- or ChABC-treatment. While it would be interesting to determine if ChABC treatment induced sprouting by other supraspinal tracts, the overall effect would be unchanged—sprouting did not appear to improve functional abilities.

These results differ from those of Onifer and colleagues. One possible reason for this is that the sprouted sensory fibers in their model were in close proximity to an appropriate target population (neurons within the DCN), thus maximizing the potential for synapse formation on a functionally relevant target. In the spinal cord, the neuronal populations are more diverse, which may hinder synapse formation if the axon is seeking a specific target.

It may be asked why ChABC treatment following spinal cord injury failed to promote functional improvement. One possible explanation is because we targeted ChABC specifically to rostral or caudal tissue, scar-associated CSPG re-

mained intact. Indeed, there was no 2B6 immunoreactivity indicative of digested CSPG at the lesion border (data not shown). Therefore, intact CSPGs were present at the injury site, which likely impeded regeneration of injured axons. Additionally, the benefits of ChABC treatment may be injury-specific and depend on which populations remain. Bradbury and colleagues (2002) hypothesized that plasticity may have a role in enhancing functional recovery following ChABC treatment of a dorsal column injury. In that instance, the only descending tract that was disrupted was the corticospinal tract. It is possible that ChABC was able to improve recovery because all of the other supraspinal tracts and virtually all of the neurons in the spinal cord remained uninjured. Another possible reason for the lack of enhanced recovery in the present study is that by limiting administration of ChABC to either rostral or caudal tissue to parse out the contributions of plasticity within either area on recovery, we affected only half of the neuronal population that mediates ChABC-induced recovery. To have any functional consequences, it may be necessary to administer ChABC both above and below the injury to promote concurrent rostral and caudal sprouting. This will be very interesting and important to pursue in the future.

The present results reaffirm those from our previous study in which increased axonal regeneration from a peripheral nerve graft "bridging" a C3 hemisection and a C5 dorsal quadrant lesion was seen in animals in which the C5 injury site was treated with ChABC (Houle et al., 2006). Regenerated axons did form putative synapses in this instance and were responsible for at least some of the functional recovery that later was diminished by severing the peripheral nerve bridge. The current study is akin to that study in the sense that ChABC was administered caudal to a hemisection injury that interrupted all ipsilateral descending fibers. Although we did not look specifically at sprouting of caudal fibers in that earlier study, the observation of sprouting in the present study with no improvement in function further supports the conclusion that the recovery seen in the aforementioned "bridging" study most likely was mediated by axons regenerating through the nerve graft and not plasticity of spared fibers.

Why did those axons regenerating beyond the nerve graft "bridge" form functional synapses whereas the sprouting axons in the current study did not? Because proper targeting is critical for functional synapse formation, it is possible that the serotonergic sprouts induced by ChABC did not encounter the proper neuronal pool on which to synapse. It is important to note that because the hemiconfusion injury model spares multiple components of descending systems we do not know how many of the increased sprouted 5HT+ fibers originated ipsilaterally or contralaterally to the lesion. Of interest is whether there are cues that impart unilateralism in the adult spinal cord that would bias synapse formation for a particular side. Additionally, it has been suggested that CSPGs play a role in proper synapse maturation during development (Yamagata and Sanes, 2005). Perhaps digestion of CSPGs with ChABC in this model hindered synapse formation. A better understanding of synapse establishment within an injured system is critical for maximizing the potential for recovery.

In conclusion, this study demonstrates that administering ChABC rostral or caudal to a unilateral spinal cord injury is sufficient to promote plasticity of supraspinal fiber populations; however, this plasticity does not appear to have

functional ramifications. These data demonstrate that sprouting rostral or caudal to a spinal cord injury does not always translate to recovery and indicate that other mechanisms may be responsible for ChABC-mediated functional recovery.

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Author Disclosure Statement

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Address correspondence to:

John D. Houle, Ph.D.

Drexel University College of Medicine

Department of Neurobiology and Anatomy

2900 Queen Lane

Philadelphia, PA 19129

E-mail: jhoule@drexelmed.edu