

## Original Article

# Calcitonin gene-related peptide immunoreactivity in chronic human spinal cord injury

AD Ackery<sup>1</sup>, MD Norenberg<sup>2</sup> and A Krassioukov<sup>\*,1,3</sup>

<sup>1</sup>International Collaboration on Repair Discovery (ICORD), Division of Physical Medicine and Rehabilitation, Department of Medicine, School of Rehabilitation, University of British Columbia, Vancouver, British Columbia, Canada; <sup>2</sup>Miami Project to Cure Paralysis, Department of Neuropathology, University of Miami, Miami, FL, USA; <sup>3</sup>Division of Physical Medicine and Rehabilitation, Department of Medicine, School of Rehabilitation, University of British Columbia, Vancouver, British Columbia, Canada

**Study Design:** Histopathological study of the human spinal cord.

**Setting:** International Collaboration on Repair Discoveries, Vancouver, BC, Canada.

**Rationale:** In animals, primary dorsal root afferent fibers, which are immunoreactive for calcitonin gene-related peptide (CGRP), sprout following spinal cord injury (SCI) into deeper laminae of the dorsal horn below the level of injury. It has been suggested that this aberrant sprouting plays a role in altering cardiovascular control after SCI and could be responsible for life-threatening episodes of autonomic dysreflexia (AD).

**Objectives:** To observe the changes of CGRP distribution after SCI and compare the differences between normal and injured human spinal cord.

**Methods:** Upper thoracic segments from individuals with chronic cervical SCI ( $n=4$ ) and individuals with intact spinal cords ( $n=5$ ) were processed immunocytochemically to identify CGRP fibers and histologically to identify the severity of degeneration.

**Results:** Semiquantitative analysis showed a significant increase in CGRP immunoreactivity in the dorsal horns of individuals with chronic SCI ( $P<0.001$ ). Furthermore, one of the SCI individuals in this study displaying significant CGRP sprouting had well documented episodes of AD.

**Conclusions:** Our observations suggest that SCI in humans results in significant sprouting of CGRP fibers. This aberrant sprouting of sensory fibers could contribute to the abnormal cardiovascular control and pain commonly observed following chronic human SCI.

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**Keywords:** calcitonin gene-related peptide; dorsal root sprouting; human; spinal cord injury

## Introduction

Spinal cord injury (SCI) results in a significant alteration of autonomic control. However, the underlying mechanisms of autonomic dysfunction following SCI are not well understood.<sup>1–3</sup> Unstable arterial blood pressure and urinary bladder dysfunctions are well-known clinical conditions in individuals with SCI requiring careful management and sometimes numerous hospital readmissions.<sup>4,3</sup> As SCI is associated with an increased risk of stroke and coronary heart disease,<sup>5–7</sup> unstable arterial blood pressure may further increase the risk of vascular injury and cerebrovascular disease in this population.<sup>8</sup> Aberrant plasticity of the spinal autonomic circuits following SCI could be one of the contributing

mechanisms leading to abnormal cardiovascular control following SCI.<sup>9–11</sup>

In animals with cervical and mid-thoracic (T6 or above) SCI, it has been shown that sprouting of primary dorsal root afferents within the dorsal horns can create exaggerated spinal reflexes that can lead to episodic hypertension, also known as autonomic dysreflexia (AD).<sup>11</sup> In humans, AD can manifest as a severely debilitating and occasionally life-threatening condition requiring immediate intervention.<sup>12–16</sup> The aberrant sprouting and formation of inappropriate connections by primary afferent fibers related to SCI are possible causes for AD and chronic pain.<sup>9,10,17</sup>

Calcitonin gene-related peptide (CGRP) is a protein found in a variety of dorsal ganglion cells, including unmyelinated afferent C-fibers and lightly myelinated afferent A $\delta$  fibers.<sup>18–20</sup> In uninjured spinal cord, CGRP

\*Correspondence: Dr A Krassioukov, ICORD, Division of Physical Medicine and Rehabilitation, 2469-6270 University Blvd., Vancouver British Columbia, Canada V6 T 1Z4

immunopositive axons convey pain and temperature information, synapsing on second order neurons within lamina I and II of the spinal dorsal horns (Figure 1).<sup>21–23</sup> These second order axons cross at the level of the spinal cord within the anterior spinal commissure and ascend through the spinothalamic tract to the thalamus. It has been shown that in animals with SCI, CGRP-containing fibers sprout into deeper laminae.<sup>11,24</sup> This sprouting magnifies the sensory afferent input of spinal circuits involved in spinal reflexes and promotes AD.<sup>11</sup> The development of AD in these animals is highly correlated with the extent of sprouting of CGRP-containing fibers. Also, experimental studies suggest that nociceptive input to the spinal cord may play an important role in the development of AD after SCI.<sup>10</sup> For example, in a rat model of chronic SCI, responses to noxious and non-noxious somatic and visceral stimuli were exaggerated. This included increased activity of spinal interneurons and sympathetic nerves, with greater increases in blood pressure.<sup>10</sup>

To date, little is known with respect to CGRP sprouting in human SCI. This study compared changes in CGRP immunoreactivity between individuals with chronic cervical SCI and individuals with an uninjured central nervous system (CNS). This study also provides a case study correlating the changes in CGRP histology in a SCI patient with well-documented cardiovascular dysfunction and documented history of AD.

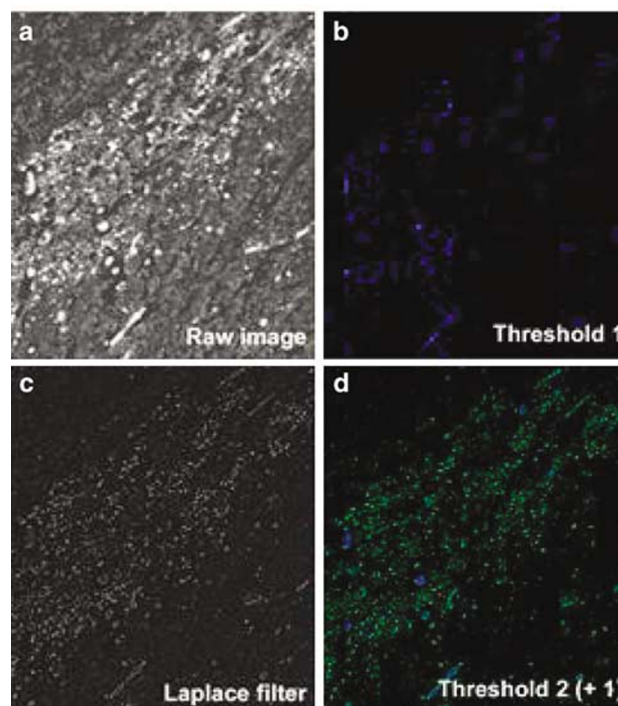
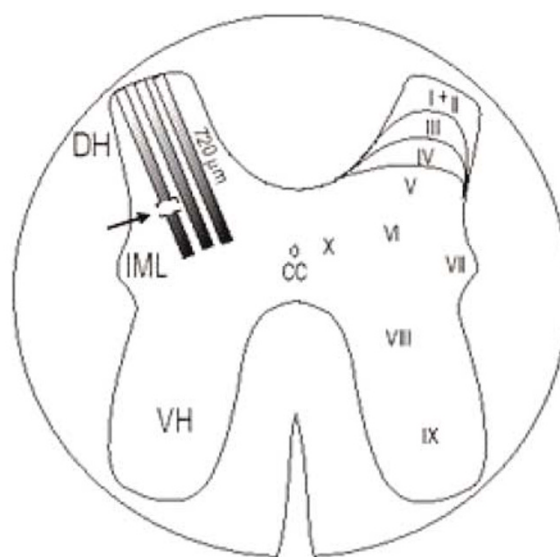
## Methods

### Human spinal cord tissue selection

The University of British Columbia Clinical Research Ethics Board has approved protocols for this study. The human tissues were selected from the collections of the Miami Project to Cure Paralysis and Toronto Western Hospital. A retrospective chart analysis was also conducted in order to collect demographic (age, gender) and clinical information (severity and level of SCI, and

other secondary complications). Spinal cord specimens of both sexes, from individuals with chronic cervical SCI ( $n=4$ ) and control case ( $n=5$ ) with intact CNS with known postmortem intervals ( $\leq 24$ h) were included in the study (Table 1).

The upper-mid thoracic segments were selected for numerous reasons. Based on our previous experimental observations in animals with complete transections, we can expect that following severe SCI in humans, the CGRP sprouting could occur in numerous segments below the level of lesion.<sup>11</sup> Secondly, we were specifically interested in thoracic segments because they are an area of localization of the spinal autonomic neurons innervating the heart and blood vessels.<sup>25</sup> Finally, individuals



**Figure 1** Top. Schematic diagram of the human spinal cord and the densitometry technique. The major anatomical landmarks are indicated on the left side of diagram: dorsal horn (DH), ventral horn (VH), intermediolateral nuclei (IML) central canal (cc) and laminae I–X. Three 50- $\mu$ m wide rectangular strips extending 720- $\mu$ m into the dorsal horn (right side of diagram) represent the computer-generated area of analysis of CGRP immunopositive fibers. A computerized program provided average density measurements for each 20- $\mu$ m segment (boxed area indicated by arrow) of the 720- $\mu$ m long rectangular strip. The average of these three measurements was plotted to create a point on the graph in Figure 3. Bottom. Densitometric analysis. From each raw image (a) a high-threshold overlay was generated (b), and saved. The raw image was then processed with a Laplacian edge-detection filter (c), after which a second, low-threshold overlay (green overlay in d) was generated. The high- and low-threshold overlays were combined and the density of immunoreactive processes was measured from the dorsal gray/white matter interface

**Table 1** Demographic and clinical data

Cases	Gender	Age	Level of SCI	ASIA	Level of CGRP staining	Cause of injury	Time from SCI to death
<i>Chronic SCI</i>							
1	Male	22	T3	A	T6	Gun shot wound	9 months
2	Male	58	C8	A	T2	Motor vehicle collision	2 years
3	Male	34	C4	Unknown	T3	Fall	17 years
4	Female	31	C2	A	T4	Diving accident	9 months
<i>Uninjured controls</i>							
5	Male	69	NA	NA	T4	NA	NA
6	Male	47	NA	NA	T3	NA	NA
7	Female	37	NA	NA	T6	NA	NA
8	Female	33	NA	NA	T4	NA	NA
9	Female	30	NA	NA	T4	NA	NA

SCI, spinal cord injury; ASIA, American Spinal Injury Association; NA, not applicable

with cervical SCI most commonly present with severe autonomic/cardiovascular disorders following SCI such as AD.<sup>1,26</sup>

Although human SCI tissue provides an important opportunity to study pathophysiology, it is important to recognize the limitations of the present study related to tissue selection. There is no standard tissue procurement and fixation procedure in collecting human tissue and this introduces technical problems with respect to tissue labeling. It has been shown that the quality of staining in fetal human tissue was influenced by the interval between death and appropriate fixation.<sup>27</sup> Thus, only tissue with an autopsy time within 24 h was selected for this study.<sup>28</sup> Furthermore, the lengthy storage of the tissue leads to decreased antigen presentation needed for immunocytochemistry. To minimize this error, this study intentionally used human tissue stored for less than 6 months.

#### *Histology and immunocytochemistry*

Tissues were fixed with 10% buffered formalin for 2 weeks and embedded in paraffin. In each SCI case, at least one spinal segment below the level of injury (upper thoracic) was selected. In control cases, corresponding spinal segments were also selected. Similar to previous studies of human spinal cord tissue, several sets of alternate spinal cord sections (thickness of 5–8  $\mu\text{m}$ ) were prepared and stained.<sup>29,30</sup> The first set of alternating paraffin sections were processed with hematoxylin and eosin and luxol fast blue for routine light microscopic evaluation (H&E, LFB). The other remaining sections were processed immunocytochemically for the identification of CGRP containing fibers. For CGRP immunohistochemistry, paraffin sections were microwaved (2  $\times$  5 min) in 10 mM citrate (pH 6.2) at low power. Sections were then blocked for 1 h with 10% normal donkey serum, and then incubated overnight with a rabbit anti- $\alpha$ -CGRP primary antibody (1:200; Peninsula Laboratories, San Carlos, CA, USA). Sections were processed with donkey anti-rabbit Alexa 488 fluorescent

secondary antibody for 2 h (1:400; Molecular Probes, Eugene, OR, USA). Slides were viewed under bright field/fluorescent microscopy (Axioscope, Zeiss; Jena, Germany) and images were captured using the Northern Eclipse imaging software (Version 6.0, Empix Imaging Inc., Mississauga, ON, Canada).

#### *Data analysis*

In the present study, a pixel threshold density technique was used to measure the distribution and intensity of CGRP immunofluorescence.<sup>31,32</sup> In order to prevent observer bias, all sections were coded in order to blind the analysis. Each photomicrograph was captured using Northern Eclipse software (Empix Neuroimaging, version 6.0) and was thresholded using an edge-detecting algorithm. This ensured that density measurements were only measuring CGRP-positive axons. In addition, Sigma Scan Pro 5 software (SPSS, Chicago, USA) was used to analyze the CGRP densities. The density of CGRP-positive axons was measured along a 50- $\mu\text{m}$  wide rectangular strip extending 720- $\mu\text{m}$  from the top of lamina I into the deeper laminas of the gray matter of the spinal cord (lamina VI). This technique was repeated three times, using parallel lines that did not overlap the same parts of the dorsal horn (Figure 1, top). In all cases, the strips were placed directly under Lissauer's tract and directed through the center of the dorsal horn. These measurements generated axon density data as a function of depth in the dorsal horn. A computerized program provided average density measurements for each 20- $\mu\text{m}$  segment of the 720- $\mu\text{m}$  long rectangular strip. To quantify CGRP-positive axon density in the spinal cord, we carried an automated thresholding procedure,<sup>31,33,34</sup> which first defines the thickest and brightest regions, and then, following application of a Laplacian omnidirectional edge-detection filter, the finer processes (Figure 1, bottom). The edge-detection filter effectively normalizes the signal-to-noise ratio such that small variations in immunoreactivity across sections are eliminated. However, as thicker processes and somata

are 'hollowed out' by the edge-detection filter, the edge-detection overlay was combined with the initial threshold overlay defining these thicker processes and somata. Densitometric measurements were made on the resulting combined threshold overlays as a function of distance from the lesion epicenter. This method of quantification measures the density of immunopositive objects independent of their individual intensities. Three alternate sections from each case were analyzed. The mean density  $\pm$  SEM was plotted against the depth within the dorsal horn (Figures 1 and 3).

Statistical analysis of density measurements between groups was performed using two-way ANOVA's followed by Tukey's *post hoc* analysis to compare individual depths between the groups. Significance was established at  $P < 0.001$  for two-way ANOVA's and  $P < 0.05$  for *post hoc* analysis.

## Results

### Study population

Details of the study population are provided in Table 1. Four individuals with chronic SCI (three male subjects and one female subject, ages 22–58 with a mean of 36 years) and five individuals with an intact CNS (two male subjects and three female subjects, ages 37–82 with a mean of 60 years) were selected. Individuals with SCI sustained either a cervical ( $n = 3$ ) or upper thoracic ( $n = 1$ ) injury. Three of these individuals had documented complete motor and sensory injury on neurological assessment using American Spinal Injury Association/International Medical Society of Paraplegia impairment scale (ASIA/IMSOP).<sup>35</sup> We were unable to retrieve the information on neurological evaluation in one individual with SCI (case no. 3, Table 1). The period of survival following initial injury ranged from 9 months to 17 years, and there were various causes of SCI (Table 1). In one SCI individual, significant cardiovascular dysfunctions were thoroughly documented throughout the post-injury period (case no. 4, discussed below).

### Histological evaluation

Histopathological examination of the site of injury revealed complete injury in one case (case no. 1) and confirmed partial preservation of the peripheral white matter at the level of injury in three other cases (data not presented). Sections of interest from the upper thoracic segments below the level of injury were examined with H&E and LFB and areas of white matter degeneration were readily identified. These areas of degeneration appeared pink in sections processed with H&E and LFB (Figure 2a–d). In spinal cord sections from individuals with chronic SCI below the level of injury, areas of degeneration were observed in different regions of the cord. In all SCI cases, Wallerian degeneration was predominantly observed in the descending autonomic and motor tracts within the lateral and anterior funiculae of the spinal cord (Figure 2b and d).

### CGRP immunoreactivity

In both intact and SCI groups, the majority of CGRP containing fibers were present within the dorsal laminae of the spinal cord (laminae I–II, Figure 2e–h). In individuals with chronic SCI, the varicosed branching fibers coursed individually or in bundles for several hundred micrometers through the deeper laminae of the dorsal horn (laminae III–V, Figure 2f and h). Occasionally, CGRP containing fibers in these individuals were observed in the deeper laminae VII and X (areas of localization of autonomic neurons, not shown).<sup>25</sup> In comparing the two groups, an overall increase in density of CGRP immunopositive axons was found in the individuals with chronic SCI (two-way ANOVA, differences between groups,  $P < 0.001$ , Figure 3a). *Post hoc* analysis showed that these chronic SCI CGRP increases were found in lamina I and II (Tukey's test; depths 20–80  $\mu$ m and 120–160  $\mu$ m,  $P < 0.05$ ) and in the deeper laminae IV and V (Tukey's Test; depths 420–460  $\mu$ m and 500–540  $\mu$ m,  $P < 0.05$ ) when compared to uninjured control spinal cords (Figures 2e–h and 3a).

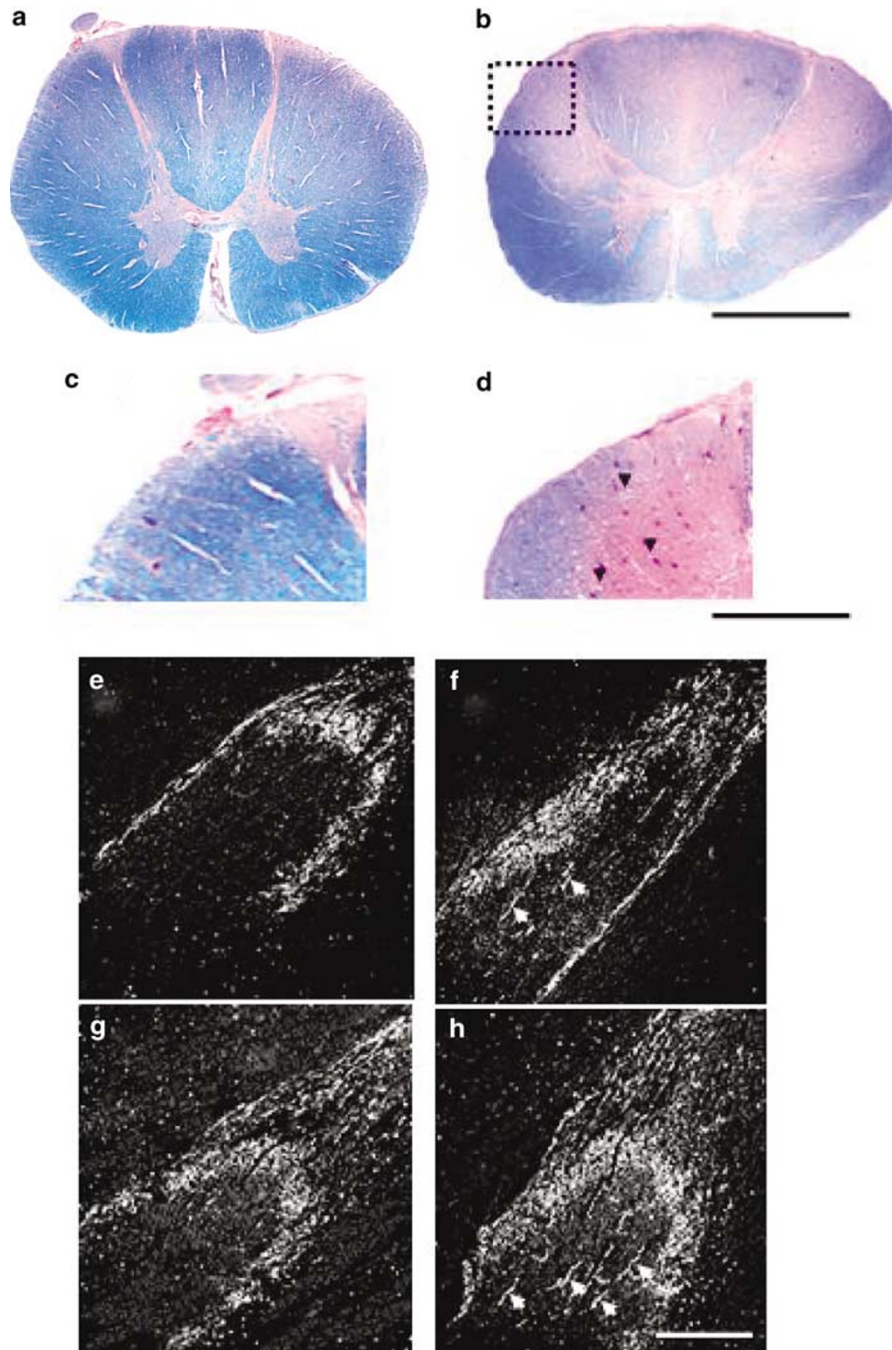
### Case study with documented AD

In case 4, additional clinical data were available to compare with our CGRP immunoreactivity. Case 4 was a 31-year-old female subject who sustained a multilevel cervical traumatic SCI as a result of a diving accident (case no. 4, Table 1). On admission, she presented with severe hypotension (neurogenic shock) requiring vaso-pressive therapy. Her neurological examination revealed a complete C2 injury (ASIA A). She underwent acute surgical decompression and spine stabilization. Her acute postoperative period and rehabilitation was complicated by severe cardiovascular dysfunction. Following her acute hypotension period, she developed numerous episodes of AD with severe hypertension and bradycardia. This patient survived 9 months following the SCI and died from respiratory complications.

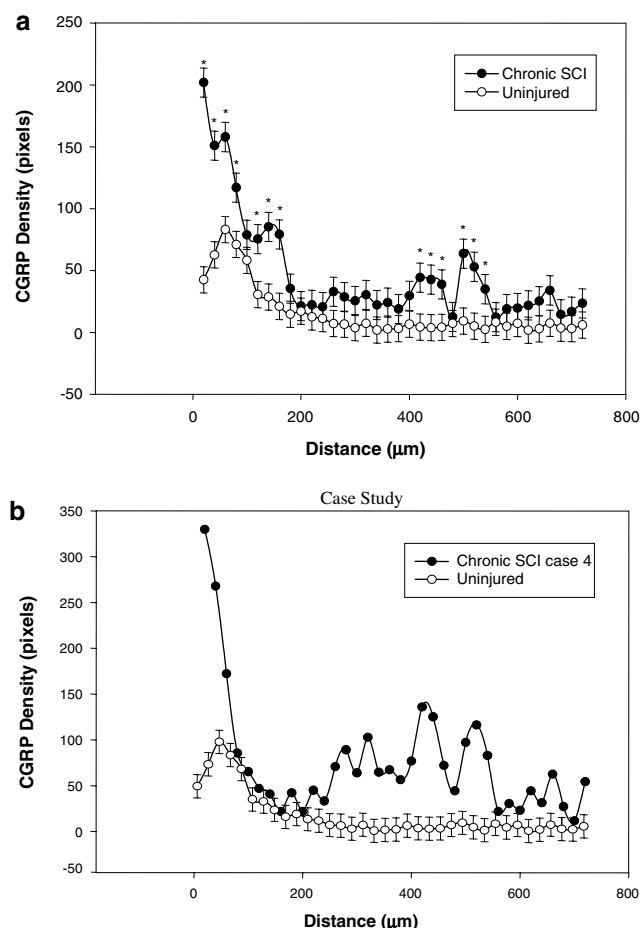
Even though this patient had complete motor and sensory deficits on her neurological exam (C2, ASIA A); the histopathology showed a partial preservation of the white matter at the site of injury. CGRP density from this case was plotted separately in order to compare the average of the CGRP immunoreactivity in uninjured control tissue (Figure 3b). Examination of CGRP containing fibers of this case exhibited a similar pattern to that of other cases of SCI. There was a noticeable increase in CGRP density in the upper laminae I and II, as well as in the deeper laminae IV–VI (Figure 3b).

## Discussion

A number of animal studies have shown that CGRP immunopositive fibers undergo sprouting within the dorsal horn following SCI.<sup>9,11</sup> It has been suggested that this aberrant sprouting and inappropriate synaptic connections within the spinal cord contribute to various conditions observed in animals following SCI: chronic pain, AD and spasticity.<sup>9–11</sup> Although numerous in-



**Figure 2** Microphotographs of spinal cord sections from individuals with intact spinal cord (**a**, **c**, **e**, **g**) and individuals with SCI (**b**, **d**, **f**, **h**). (**a–d**) Sections processed with H&E/LFB and visualized under light microscope. Well-defined butterfly shaped areas of gray matter can be observed in (**a** and **b**). Myelin containing white matter is stained blue. In (**b**) there are areas of axonal degeneration and myelin loss (pink areas within the white matter). Boxed areas within dorsolateral funiculus from (**a** and **b**) are presented in (**c** and **d**) at higher magnification. In addition to myelin loss, there are numerous vacuoles (arrowheads in **d**) that can be identified within this area, suggesting degenerative changes and axonal loss. (**e–h**) Sections processed for CGRP immunoreactivity and visualized under fluorescent microscope. In intact spinal cords, CGRP-containing fibers were localized predominantly within the dorsal root entry zone and laminae I–II. Only a few CGRP fibers were found in deeper laminae of the dorsal horns in uninjured spinal cords (**e**, **g**). In sections from individuals with injured SCI, a significant number of CGRP-containing fibers were present in the deeper laminae of the dorsal horns (**f**, **h** arrows). Calibration bars: **a** and **b** = 2 mm; **c** and **d** = 200  $\mu$ m; **e–h** = 500  $\mu$ m



**Figure 3** (a) Quantitative analysis of CGRP density in individuals with chronic SCI and uninjured controls (mean  $\pm$  SEM). (b) CGRP density from case no. 4 plotted with the average of CGRP density of uninjured group. Mean  $\pm$  SEM only included for the control group ( $n=5$ ). \*Denotes a statistical significance of  $P<0.05$

vestigations of human tissue with SCI were undertaken, little is known about the changes in CGRP fibers and their role in autonomic dysfunction following SCI in humans.<sup>36–38,35,39</sup> Our study examined, in a semiquantitative manner, changes in CGRP containing fibers from individuals with chronic SCI, and correlated these results with their clinical presentations. The data provide evidence that CGRP containing dorsal root afferents undergo sprouting in individuals with chronic SCI, and thereby extend similar data from animal observations to actual human injury. Furthermore, one case associated with well-documented autonomic disturbances displayed significant CGRP immunopositive sprouting.

#### Cardiovascular dysfunction in chronic SCI

There are at least three components of spinal autonomic circuits that play a role in abnormal cardiovascular

control following SCI: (1) descending vasomotor pathways, (2) sympathetic preganglionic neurons and (3) spinal afferents.<sup>29</sup> Following SCI, numerous plastic changes occur within these circuits resulting in either periods of low or increased sympathetic tone.<sup>29,40</sup> Low arterial blood pressure in the acute stage of SCI (neurogenic shock) and persistent orthostatic hypotension in chronic stages of SCI contribute to the loss of the descending tonic influences from the supraspinal cardiovascular pathways.<sup>29,40</sup> Periodic episodes of hypertension in individuals with SCI, known as AD, can elevate blood pressure up to 300 mmHg, resulting in life-threatening myocardial infarctions and cerebral hemorrhages.<sup>8</sup> AD can be triggered by noxious and non-noxious stimuli below the level of injury, such as bowel and bladder distension, pressure sores, spasticity and even by simple touch, resulting in an overall increased sympathetic activity.<sup>1,41–43</sup> Clinically, AD is accompanied by severe headaches, profuse sweating, piloerection, facial flushing, blurred vision and stuffy noses.<sup>3</sup> In some individuals with SCI, AD can be asymptomatic.<sup>44</sup> It became evident from animal experiments that AD results from exaggerated spinal circuits.<sup>10,45</sup> The loss of the descending inhibition and the sprouting of the dorsal root sensory afferent fibres that directly synapse on interneurons and/or preganglionic sympathetic neurons are likely to be responsible for this phenomenon.<sup>10,11,46,47</sup>

Although in the majority of human SCI the spinal cord continuity is partially preserved, it has been shown that neurologically complete SCI (ASIA A) have a higher incidence of AD, when compared to incomplete SCI.<sup>48</sup> The presently used ASIA/IMSOP scale for the neurological assessment of individuals with SCI evaluates only motor and sensory pathways within the spinal cord.<sup>49</sup> Unfortunately, ASIA/IMSOP examination does not assess the integrity of the autonomic pathways involved in cardiovascular control.<sup>50,51</sup> A recent study examined histopathological changes within these pathways in individuals with SCI and correlated the changes with neurological assessments (ASIA/IMSOP scale) and severity of cardiovascular dysfunction.<sup>29</sup> The findings suggest that individuals with severe SCI and significant destruction of the descending autonomic pathways suffered a higher degree of cardiovascular complications following SCI.<sup>29</sup> In the present study, with the exception of case no. 3, neurological assessment of individuals revealed complete SCI (ASIA A), suggesting the possibility of these individuals developing severe cardiovascular dysfunction.<sup>1,52</sup> One limitation of this study was the inability to obtain the complete follow-up clinical information on cardiovascular control. However, in one individual (case no. 4) with neurologically complete SCI (ASIA A), prolonged neurogenic shock and numerous well-documented episodes of AD were present.

#### Primary afferents containing CGRP following SCI

A variety of primary dorsal root afferents contain CGRP.<sup>18,21,53</sup> However, only unmyelinated C and

lightly myelinated A $\delta$  axons express the tropomyosin receptor kinase A, which has high affinity for nerve growth factor (NGF).<sup>54</sup> It is generally accepted that these CGRP-containing fibers are involved in nociception and transmission of painful information to the neurons within the spinal dorsal horn.<sup>53</sup> Numerous studies provide evidence that NGF is responsible for the plasticity of primary afferents containing CGRP.<sup>55,56</sup> Although NGF levels have been shown to be minimal in uninjured spinal cord, the level of NGF increased dramatically following experimental SCI.<sup>57</sup> Seven days following SCI, the NGF at the site of injury is at a maximum level and significantly increased throughout the entire spinal cord. The introduction of exogenous NGF also has been demonstrated to induce sprouting in CGRP fibers in the dorsal horn.<sup>9,58</sup> However, intrathecal therapy with anti-NGF antibody in animals with SCI was associated with the blockade of CGRP sprouting and an altered physiological response to visceral stimulations. AD, which is well developed in rats 2 weeks following spinal cord transection, was markedly reduced by this treatment.<sup>11,59</sup> Unfortunately, little is known about the changes in NGF in human spinal cord following injury.

The present study examined tissue only from spinal segments below the level of injury. It was expected that similar to animals, the changes in CGRP-containing fibers would be observed not only at the level of injury, but also in segments below the injury site.<sup>11,57</sup> The pattern of CGRP-containing fibers in intact human spinal cord was similar to that described in animals. Fibers entered through the dorsal root entry zone with higher density within the superficial laminae (I and II) of the dorsal horn. Only small amounts of CGRP-containing fibers were present in deeper lamina of the dorsal horns in individuals with intact CNS. In contrast, in individuals with chronic SCI, CGRP fibers were present in deeper laminae of the dorsal horns (laminae III–V). They often took the form of thick bundles of fibers that coursed along the medial edge of the gray matter. Similar to our observations in animals, in this study, in human spinal cord, we were not able to observe any consistent pattern of CGRP sprouting fibers. Moreover, semiquantitative assessment of CGRP fibers in individuals with SCI showed significant increases in CGRP immunoreactivity within the deeper regions (400–550  $\mu$ m) of the dorsal horns (Figure 3a).

Previously, Christensen and Hulsebosch<sup>9</sup> demonstrated the functional relevance of such sprouting. They noted that in animals with SCI, CGRP sprouting was associated with allodynia and hyperalgesia. It has also been shown that the activity of spinal interneurons, the sympathetic activity and the blood pressure response to noxious and non-noxious stimuli were exaggerated in animals with chronic SCI.<sup>10</sup> It is especially noteworthy that in rats with SCI, the activity of the sympathetically correlated spinal interneurons within one spinal segment localized in deeper laminae of the spinal cord (T10) was increased by stimulation of more of the body surface. This is consistent with the sprouting of the dorsal root

cutaneous afferents following SCI and the formation of new synaptic connections.<sup>10</sup> The data presented here suggest that CGRP-containing fibers in human spinal cord sprout following SCI, and extend beyond lamina I and II into the deeper lamina of the spinal cord. This plastic change would likely result in new inappropriate synaptic connections, and provide increased sensory input into the spinal circuits following SCI. In addition to CGRP sprouting, individual with SCI (case no. 4) presented with numerous well-documented episodes of AD, suggesting a possible link between altered function and sprouting.

We would also like to acknowledge that in addition to sprouting of CGRP-containing fibers, other mechanisms could be responsible for the observed increase in CGRP immunoreactivity in human tissue. For example, there are a few reports suggesting that in animals, large DRG neurons, which do not normally produce CGRP, begin to express it following peripheral nerve injury.<sup>56,60,61</sup> Such phenotypic switching within the DRG following peripheral nerve injury or inflammation should be also considered and will require further investigations.

## Conclusions

Much of the present understanding of the pathophysiology of CNS disorders, including SCI, is based on extrapolations from animal models. Therefore, it is essential to verify that human and animal CNSs undergo similar structural changes in normal and diseased states. The results of this study reaffirm previous results found in animal models of SCI, that CGRP-containing dorsal root afferents sprout following human SCI. This aberrant sprouting may contribute to cardiovascular dysfunction and the development of life-threatening episodes of AD. This human data, in addition to previous observations in animal models, reveals a likely mechanism for the development of AD and a possible strategy for its prevention. Developing treatments, targeted to neutralize the intraspinal effects of NGF and prevent the sprouting of CGRP-containing afferent fibers, could impede the development of AD and markedly improve the quality of life of individuals with SCI.<sup>11</sup>

However, we also have to be cautious with the use of therapeutic trophic factors, which have been used to promote the regeneration of the spinal pathways following SCI. We have to be aware that in addition to their beneficial effects, regeneration paradigms could lead to severe side effects, such as aberrant sprouting and inappropriate connections within the spinal cord. These well-intended therapeutic approaches could result in greater abnormal cardiovascular responses and exaggerated states of pain.<sup>10,62</sup>

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