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Mechanisms of Chronic Central Neuropathic Pain after Spinal Cord Injury

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Abstract

Not all spinal contusions result in mechanical allodynia, in which non-noxious stimuli become noxious. The studies presented use the NYU impactor at 12.5 mm drop or the Infinite Horizons Impactor (150 kdyne, 1 sec dwell) devices to model spinal cord injury (SCI). Both of these devices and injury parameters, if done correctly, will result in animals with above level (forelimb), at level (trunk) and below level (hindlimb) mechanical allodynia that model the changes in evoked somatosensation experienced by the majority of people with SCI. The sections are as follows: 1) Mechanisms of Remote Microglial Activation and Pain Signaling in “Below-Level” Central Pain 2) Intracellular Signaling Mechanisms in Central Sensitization in “At-Level” Pain 3) Peripheral Sensitization Contributes to “Above Level” Injury Pain Following Spinal Cord Injury and 4) Role of Reactive Oxygen Species in Central Sensitization in Regional Neuropathic Pain Following SCI. To summarize, differential regional mechanisms contribute to the regional chronic pain states. We propose the importance of understanding the mechanisms in the differential regional pain syndromes after SCI in the chronic condition. Targeting regional mechanisms will be of enormous benefit to the SCI population that suffer chronic pain, and will contribute to better treatment strategies for other chronic pain syndromes.

Keywords

Reactive Oxygen Species; cytokines; Central Sensitization; Peripheral Sensitization; Pain; Chronic Pain; Regional Pain Syndrome

Introduction

Chronic Pain and Spinal Cord Injury

Spinal cord injury (SCI) is a devastating event that results in motor dysfunction below the level of lesion, as well as the development of chronic pain syndromes. There are approximately 400,000 spinally-injured patients in the U.S. with over 14,000 new injuries occurring each year (Sekhon and Fehlings, 2001). In both complete and partial spinal lesions, chronic pain develops

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within months following injury (Richards et al., 1980). Up to 80% of patients experience clinically significant pain which is described as burning, stabbing, and/or electric-like (Finnerup et al., 2001; Siddall et al., 2003). Post-SCI pain can produce drastic impairments in daily routines and quality of life to a greater extent than motor impairment (Rintala et al., 1998), and is refractory to clinical treatments despite a variety of neurosurgical, pharmacological, and behavioral therapeutic strategies (Balazy, 1992; Turner et al., 2001). The pain so greatly affects the quality of life that depression and suicide frequently result (Cairns et al., 1996; Segatore, 1994).

The pain syndromes are permanent and, because the lesions are central, are referred to as central neuropathic pain (CNP). Research focused on improving recovery of function, including the reduction of CNP, is essential. The pain syndromes or dysesthesias (disturbing somatic sensations that may not be painful) can be divided into two broad categories based upon their dependency or independence of peripheral stimuli: 1) spontaneous pain – which occurs independently of peripheral stimuli, is persistent, waxes and wanes intermittently, and is described as numbness, burning, cutting, piercing or electric-like (Davidoff and Roth, 1991); 2) peripherally evoked pain – which occurs in response to either normally nonnoxious or noxious stimuli. In addition, some chronic spinal cord injured patients experience a band or “girdle” of hyperpathia and/or allodynia at the level of the sensory loss (Tasker and Dostrovsky, 1989). Siddall and colleagues (2002) defined three regions of pain that result from SCI: 1) above-level pain which occurs at dermatomes cranial to the injury site, 2) at-level pain which occurs in dermatomes near the spinal injury, develops shortly after SCI, where the pain is often characterized as either stabbing or is a stimulus-independent type that is accompanied by allodynia, and 3) below-level pain, which is localized to dermatomes distal to the injury site, develops more gradually than at-level pain, and is often classified as a stimulus independent continuous burning pain (Sjolund 2002; Vierck Jr et al. 2000). We have experience testing evoked pain and have pioneered methods for measuring spontaneous pain in animals (Hulsebosch et al., 2000; Mills, et al., 2001a).

Modeling Central Neuropathic Pain

Little attention has been given to mechanisms of chronic pain in SCI in the clinics, and it has only been in the last several years that animal models were developed to study the development and maintenance of CNP-like behavior after SCI. The models include an intravascular photochemical reaction that occludes blood vessels, producing spinal cord ischemia with subsequent trunk mechanical allodynia (Hao et al., 1991; Xu et al., 1992); anterolateral lesions of the spinal cord in monkeys and rats that produce overgrooming and mechanical allodynia (Ovelmen-Levitt, et al., 1995; Vierck and Light, 2000); a clip compression model in which the thoracic spinal cord is compressed by a 35 g or 50 g clip that results in mechanical hyperalgesia in the hindlimbs (Bruce et al., 2002); quisqualic acid injection (an AMPA/kainate and metabotropic receptor agonist) into the dorsal horn that produces overgrooming (Yeziarski, et al., 1998); a spinal hemisection model of CNP (Christensen et al., 1996; Christensen and Hulsebosch, 1997; Gwak et al., 2008); and a spinal contusion model, in which mechanical allodynia “girdles” the trunk (Siddall et al., 1995; Lindsey, et al., 2000; Hulsebosch, et al., 2000). We have further characterized this last model, demonstrating the presence of mechanical and thermal allodynia in both forelimbs (above level), “girdling” (at level) and in both hindlimbs (below level) (Hulsebosch et al., 2000). The spinal contusion model best parallels the injury profile described in human spinal cord injury (Bunge et al., 1993; Bunge, 1994).

The following four sections focus on mechanisms of central neuropathic pain in the spinal contusion model in the rat. Not all spinal contusions result in mechanical allodynia (i.e., the phenomenon in which non-noxious stimuli become noxious). The studies presented use the NYU impactor at 12.5 mm drop or the Infinite Horizons Impactor (150 kdyne, 1 sec dwell).

Both of these devices and injury parameters, if done correctly, will result in animals with above level (forelimb), at level (trunk) and below level (hindlimb) mechanical allodynia that model the changes in evoked somatosensation experienced by the majority of people with SCI. The sections are as follows: 1) Mechanisms of Remote Microglial Activation and Pain Signaling in “Below-Level” Central Pain (Bryan C. Hains); 2) Intracellular Signaling Mechanisms in Central Sensitization in “At-Level” Pain (Eric D. Crown); 3) Peripheral Sensitization Contributes to “Above Level” Injury Pain Following Spinal Cord Injury (Susan M. Carlton) and 4) Role of Reactive Oxygen Species in Central Sensitization in Regional Neuropathic Pain Following SCI (Claire E. Hulsebosch).

1. Mechanisms of Remote Microglial Activation and Pain Signaling in “Below-Level” Central Pain

Dorsal Horn Neuron Hyperexcitability and Central Sensitization

After SCI, when the quality of a peripheral stimulus does not change, central mechanisms must account for the observed enhancements in nociceptive processing of dorsal horn neurons (but see below). SCI results in sustained hyperexcitability of these neurons, the majority of which comprise the spinothalamic tract (STT). Extracellular recordings from the dorsal horn in various models of acute and chronic SCI reveal changes in electrophysiologic properties (Hao et al., 1992; Yeziarski and Park, 1993; Drew et al., 2001; Hains et al., 2003a,^b; Hains and Waxman, 2006; Lampert et al., 2006) including shifts in proportions of neurons responding to noxious stimulation, increased and irregular spontaneous background activity, increased evoked activity to (formerly) innocuous and noxious stimuli, and alterations in sodium currents. Observations in rodent models are analogous to the abnormal functional characteristics of spinal neurons in SCI patients with chronic pain (Loeser et al., 1968).

Microglial Activation and Pain Modulation after SCI

Until very recently, pain resulting from injury to the nervous system was thought to arise primarily from the dysfunction of neurons. However, recent evidence indicates that alterations in the neuroimmune system also contribute to chronic pain. A unique class of neuroimmune cells called microglia, which normally exist in a resting but sensing state, shift their status and undergo transformation to more reactive (or “alerted” or “activated”) states in response to pathology or injury (Hanisch and Kettenmann, 2007). Microglia are implicated in the initiation phase of peripheral injury-induced pain (Coyle, 1998; Fu et al., 1999; Watkins et al., 2001; Jin et al., 2003; Ledebor et al., 2005; DeLeo et al., 2006; Watkins et al., 2007), and are activated via local release of factors such as IL-6, ATP, substance P, CCL2, and fractalkine (Ji and Strichartz, 2004; Marchand et al., 2005; Wieseler-Frank et al., 2005; Moalem and Tracey, 2006).

Microglia are also chronically activated in the spinal cord after clinical (Schmitt et al., 2000; Chang, 2006) and experimental SCI (Hains et al., 2003c; Sroga et al., 2003; Nesic et al., 2005; Zai and Wrathall, 2005; Crown et al., 2006; Hains and Waxman, 2006; McKay et al., 2007; Zhao et al., 2007a). We recently showed that there is a dramatic shift in microglial status from a resting to an activated state in the lumbar spinal cord after SCI at a time when dorsal horn nociceptive neurons fire at very high rates in response to stimulation of peripheral receptive fields of the skin, and pain-related behaviors such as mechanical allodynia and thermal hyperalgesia are evident (Hains et al., 2003c; Hains and Waxman, 2006). Selective pharmacological inhibition of microglial activation and signaling results in a return to the resting morphological phenotype as well as reductions in electrophysiologic and behavioral concomitants of pain (Hains and Waxman, 2006; Zhao et al., 2007a,b). We recently identified a mechanism by which microglia activated after SCI utilize PGE₂ as a signaling molecule to induce dorsal horn sensory neurons to undergo changes in excitability that underlies chronic

pain (Zhao et al., 2007a). We further demonstrated that within activated microglia, PGE₂ production and release is regulated by pERK1/2 MAP kinase (Zhao et al., 2007a). This is mechanistically different than from peripheral nerve injury where microglia play a role in the initiation phase of pain.

CCL21 as a Novel Modulator of Microglial Activation

Rapid morphological transformation and homing of microglia to sites of injury is induced by locally upregulated chemokines (Streit et al., 1999; Columba-Cabezas et al., 2003; Carbonell et al., 2005; Kurpius et al., 2006). Chemokines activate specific receptors on microglia to trigger activation (White et al., 2005a; Watkins et al., 2007). Our data identifies a new neuron-microglia signaling mechanism involving the cysteine-cysteine chemokine ligand 21 (CCL21, Exodus-2, 6Ckine) which is upregulated after nervous system injury. Until now, only fractalkine (CX3CL1) and CCL2 (MCP-1) have been implicated in microglial activation. Fractalkine is a potent microglial modulator after peripheral nerve injury and contributes to the induction phase of pain (Milligan et al., 2004; Verge et al. 2004). CCL2 is upregulated in damaged dorsal root ganglion neurons (White et al., 2005b), and is transported to sensory terminals in the spinal cord where it activates microglia (Zhang and DeKoninck, 2006).

CCL21 elicits a chemotactic response by microglia but not monocytes or neutrophils (Gunn et al., 1999), that can be disrupted in CCL21 receptor knock-out mice (Rappert et al., 2004). Diffusible CCL21 binds and activates the CCR7 and CXCR3 receptors expressed by microglia (Soto et al., 1998; Dijkstra et al., 2006). Microglia activated by LPS stimulation *in vitro* (Dijkstra et al., 2006), in MS lesions (Serafini et al., 2006), and in models of EAE during symptom onset and progression (Columba-Cabezas et al., 2003; Dijkstra et al., 2006), upregulate the CCR7 receptor. Microglia respond to CCL21 via lower-affinity activation of the CXCR3 receptor (Murphy et al., 2000; Rappert et al., 2002; Dijkstra et al., 2004). As in the thalamus after SCI (Zhao et al., 2007b), intraspinal injections of recombinant CCL21 robustly elicits microglial activation, neuronal hyperexcitability, and mechanical allodynia. Additionally, CCL21 neutralization reverses these pain-related phenomena after SCI.

CCL21 Induction by Massive Glutamate Release after SCI

Both CCL21 upregulation and release are observed in an *in vitro* model of neuronal injury where neurons are exposed to high concentrations of glutamate (de Jong et al., 2005). In rodents, SCI leads to dramatic (up to 50-fold) increases in local and regional (up to 5 mm away from the injury site) tissue levels of the excitatory amino acids glutamate and aspartate (Liu et al., 1999; McAdoo et al., 1999). This region encompasses the ventrolateral quadrant of the spinal cord that contains the STT. The STT carries nociceptive information supraspinally. CCL21 could thus be upregulated in the cell bodies of STT neurons whose axons have been damaged by injury-associated events after SCI. Indeed, CCL21 is synthesized by damaged nociceptive neurons in response to high levels of excitatory amino acid release after SCI, and distal release of CCL21 triggers microglial activation at spinal segments far from the site of injury. Similar microglial responsiveness to damaged neurons is observed during neurodegeneration (Sørensen et al., 1996; Vela et al., 2002). Levels of CCL21, a potent microglial activator, are dramatically increased in the lumbar enlargement as a result of thoracic SCI. It is likely that CCL21 is acting as a remote modulator and/or trigger of microglial activation. In this case, the axons of STT neurons that originate in the lumbar enlargement are damaged as they pass by the SCI site, triggering CCL21 upregulation and release at the cell bodies in the lumbar enlargement.

2. Intracellular Signaling Mechanisms in Central Sensitization in At-Level Pain

Research involving the examination of below level pain has led to the elucidation of a number of mechanisms that may contribute to the development and maintenance of chronic pain including: 1) increased sensitivity due to loss of nerve input (Nakata et al. 1979; Wright and Roberts 1978); 2) removal of inhibitory influences (Devor and Wall 1981; Hains et al. 2002; Lombard et al. 1979); 3) increased efficacy of previously ineffective synapses (Basbaum and Wall 1976; Devor and Wall 1981); 4) deafferentation hyperexcitability of spinal and/or thalamic neurons (Lenz et al. 1994; Rinaldi et al. 1991); 5) development of abnormal ion channels that alter the membrane properties of pain cells (Waxman 2001); 6) alterations in transporter distribution and activity, such as the SCI-induced reversal of glutamate transporters that leads to increases in extracellular glutamate (Vera-Portocarrero et al. 2002) and 7) transmitter and/or receptor plasticity (Hains et al. 2002; Mills and Hulsebosch 2002a). Despite the scientific gains in our understanding of below level pain, research is lacking on the role these mechanisms play in the other categories of neuropathic pain after SCI. We have chosen to focus on at-level pain that occurs rostral to a contusion injury, in the hopes of furthering our understanding and creating better ways to alleviate pain following SCI. This aim has been supported by the recent development of a model (Hulsebosch et al. 2000; Lindsey et al. 2000) in which rats given a contusion injury develop at-level mechanical allodynia by 30 days after injury.

Recent research suggests neuropathic pain that develops after peripheral injury (e.g., capsaicin injection, formalin injection, or sciatic nerve injury) is a result of central sensitization. Central sensitization refers to persistent hyperexcitability observed in dorsal horn neurons after insult and shares a number of molecular similarities with the phenomenon of long-term potentiation (a phenomenon believed by many to underlie the synaptic changes associated with learning and memory). For example, both phenomena have an early activity-dependent phase that alters neuronal excitability through synaptic strengthening and a later transcription-dependent phase that relies on the formation of new proteins (Ji et al. 2002, 2003; Nguyen and Kandel 1996). In addition, both the late phase of hippocampal LTP and transcription-dependent central sensitization have been linked to initial activation of the NMDA receptor, followed by subsequent activation of downstream intracellular enzymatic cascades involving adenylyl cyclase, PKA, PKC, and/or CaMK (Roberson et al. 1999). During both phenomena, these cascades can also be induced by intracellular increases in calcium, cAMP, nerve growth factor, nitric oxide, or CaMKII (Ginty et al. 1994; Impey et al. 1999; Sheng et al. 1991; Sweatt 2001; Roberson et al. 1999). Activation of these cascades leads to activation (via phosphorylation) of a number of MAPKs, including extracellular signal related kinase (ERK) 1/2, c-Jun N terminal kinase (JNK), and p38 MAPK which then, in turn, can lead to phosphorylation of transcription factors such as cyclic AMP responsive element binding protein (CREB) and changes in gene transcription (Lonze and Ginty 2002). Whereas evidence exists for changes in expression of long-term potentiation related mitogen-activated protein (MAP) kinases and transcription factors following peripheral injury (for review, see Ji and Woolf 2001 and Ji and Suter 2007), this hypothesis has yet to be tested rigorously in a model for chronic central pain and SCI. It is well known that SCI causes an increase in excitatory amino acid concentrations that lead to increases in intracellular calcium levels (Faden and Simon 1988; Liu et al. 1991; McAdoo et al. 1999; Tator and Fehlings 1991). Given these and other data, our hypothesis was that increases in intracellular calcium levels could trigger the activation of a number of cell signaling cascades that cause the development and contribute to the maintenance of at-level neuropathic pain.

Our first goal in testing this hypothesis was to establish that central neuropathic pain following spinal cord injury was related to increased activation of intracellular signaling kinases and

transcription factors that are associated with central sensitization. To accomplish this goal, Sprague-Dawley rats were given a contusive spinal cord injury at thoracic level T10 and then monitored weekly for the development of at-level neuropathic pain. The model we developed to study at-level neuropathic pain involves the application of von Frey filaments to the rat's back in the region at and around the site of injury. Using this model, changes in the percentage of vocalization responses to the application of force were used as the primary measure of the development of at-level neuropathic pain. In our first study using this paradigm (Crown et al 2005), we found that rats given moderate contusion injury (12.5 mm drop using the NYU impactor) developed at-level neuropathic pain by 35 days post injury. This neuropathic pain was not evident until at least 14 days post injury. After testing the rats at 35 days post injury, spinal cord tissue was taken from the site of injury to examine changes in the activation state of the transcription factor CREB at the level of the injury. Compared to sham and naïve rats, SCI rats were found to have significant increases in the expression of the activated form of CREB (pCREB). This activation was seen throughout the thoracic spinal cord and also was found to occur within spinothalamic tract cells in the thoracic dorsal horn.

The next research question we sought to answer was whether increased pCREB activation was a general result of spinal cord injury or whether this increased activation was specific to SCI rats that developed neuropathic pain. As discussed above, not all humans that received a spinal cord injury develop at-level neuropathic pain. This same variability occurs in rodents and is believed to be related to the severity of injury, as more severe spinal cord injuries produce a greater percentage of rats that develop neuropathic pain. Using an injury severity that causes ca. 50% of rats to develop at-level neuropathic pain, we were able to examine 2 separate populations of rats, those that developed neuropathic pain and those that did not. Spinal cord tissue at and around the site of the contusion injury was taken from rats at 35 days post injury, at a time point when at-level neuropathic pain had been established. As hypothesized, the results from this series of experiments indicated that increased pCREB activation occurred in SCI rats that developed at-level neuropathic pain, whereas SCI rats that failed to develop neuropathic pain were not different than sham or naïve rats (Crown et al 2006). In addition, we also examined whether kinase activation upstream of pCREB activation was increased specifically in SCI rats that developed at-level neuropathic pain. These experiments found that increased activation of ERK1/2, p38 MAPK, and CaMKII (but not JNK) occurred in rats that developed at-level neuropathic pain relative to the other groups. Taken together, these data indicate that persistent activation of intracellular signaling cascades involving MAP kinases and the transcription factor CREB are related to the maintenance of at-level neuropathic pain after SCI (Figure 2). We have recently also begun to use pharmacological tools designed to inhibit the activation of intracellular kinases to examine whether MAP kinase activation is causally related to at-level neuropathic pain. Using the inhibitor of p38 MAP kinase, SB203580, we have shown that inhibiting this kinase can attenuate the expression of at-level neuropathic pain (Crown et al, 2008). Future work will continue to examine the role of different intracellular kinases in the development and maintenance of at-level neuropathic pain.

In summary, the data indicate that activation of MAP kinases and the transcription factor CREB, are important mediators of the intracellular events that lead to the development of persistent hyperexcitability in the dermatomes corresponding to the site of injury. Further work is needed to examine fully the role of these important signaling molecules in at-level neuropathic pain.

3. Peripheral Sensitization Contributes to “Above Level” Injury Pain Following Spinal Cord Injury

Central Sensitization After SCI

The hyperexcitability and increased sensitivity of dorsal horn neurons to sensory stimuli (cutaneous, musculoskeletal, visceral, etc) has been referred to as central sensitization (Woolf, 1983; Willis, 1993a; Hulsebosch, 2003). One mechanism by which central sensitization can be achieved in the intact spinal cord is through increased peripheral input, particularly C input, as seen following capsaicin injection (Willis, 2001), peripheral injury (Woolf, 1993; Ren and Dubner, 1999), or inflammation (Neugebauer et al., 1994). The changes observed in sensitized dorsal horn cells parallel changes observed in pain states, as described in both rodent and primate models of peripheral neuropathy (Palecek et al., 1992a,b), arthritis (Neugebauer et al., 1994) and intradermal capsaicin (Willis, 1993b; Zhou et al., 2000). We and others have demonstrated central neuropathic pain behaviors after SCI that correlate with the presence of dorsal horn hyperexcitability (Christensen and Hulsebosch, 1997; Hains, 2003a,b; 2003; Drew et al., 2001). Thus, SCI can also lead to central sensitization by mechanisms similar to peripheral nerve injury (Yeziarski, 2000; Hulsebosch, 2003). For example, after SCI, there is a 37 fold increase in extracellular glutamate (Liu and McAdoo, 1993a). The increased concentrations of glutamate in the spinal cord activate glutamate receptor mediated biochemical pathways in dorsal horn neurons resulting in central sensitization. Similar events follow peripheral nerve injury, C fiber stimulation, or capsaicin injection (Yeziarski, 2000; Hulsebosch, 2003). However, the central sensitization after SCI in the rodent contusion model persists for life (Hulsebosch et al., 2000; Hulsebosch, 2005), unlike that seen in peripheral injury models in which pain persists for weeks at most. On the basis of these findings, it is important to determine at a cellular level the temporal changes in properties of dorsal horn neurons in different regions of the spinal cord following spinal cord contusion and the intracellular signaling involved.

A series of experiments focused on characterized cells in the dorsal horn that demonstrate hyperexcitability after SCI. Dorsal horn neurons were examined that respond to a range of both noxious and non-noxious stimuli, wide dynamic range (WDR) neurons, low threshold (best response to low threshold stimulus, LT) and high threshold (best response to high threshold stimulus, HT) neurons. In neuropathic pain models, dorsal horn neurons are characterized by their responses to somatic stimuli. In addition, the background activity is determined, and the receptive field is mapped. In general, when there is central sensitization, WDR cells have increased background activity, expanded receptive fields, increased after discharge rates, and increased responses to normally nonpainful mechanical stimuli (Christensen and Hulsebosch, 1997; Hains, et al., 2003a,b). The mechanisms that have been proposed to lead to central sensitization are listed above. Ways to prevent central sensitization as a result of SCI have not been fully explored. One goal of this study is to investigate the contributions of peripheral sensitization to altered sensory experience after SCI, particularly on above the spinal lesion level. In this region, release from descending inhibition, alterations due to injured peripheral components (ex. dorsal root injury) or injury to collateral central projections can not account for the altered conduction properties reported in the nerves of the forelimb.

Peripheral Sensitization After SCI

Following thoracic SCI, our preliminary data demonstrate peripheral sensitization of nociceptors in the glabrous skin of the forelimb (above level) but not in the hindlimb (below level). Peripheral sensitization is characterized by a lowering of the threshold to mechanical and heat stimuli and/or an increase in response to these stimuli (Carlton et al. 2001a,b; Du et al. 2001,2003). Although the process is not completely understood, sensitization can occur following activation of membrane receptors which are coupled to intracellular signal

transduction cascades (Guenther et al 1999). Protein kinases in these cascades then phosphorylate molecules in the cytoplasm or ion channels and receptors in the membrane. The result is a lowered threshold to activation and/or an increased response to stimulation, known as peripheral sensitization (Lopshire and Nicol, 1998). Whereas it is commonly held that peripheral changes lead to central sensitization, paradoxically, changes in the chemical milieu in the spinal cord can lead to sensitization of primary sensory neurons in a retrograde fashion. For example, intrathecal injection of glutamate, NMDA, or prostaglandin E₂ (PGE₂) can cause mechanical hyperalgesia which can be attenuated by intraplantar injection of either morphine or the NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) (Ferreira and Lorenzetti, 1994, 1996). The authors hypothesize that once central terminals of primary afferents are sensitized, the change in membrane properties are rapidly extended by some unknown mechanism, resulting in “retrograde sensitization” of peripheral terminals of primary afferent. Reactive oxygen species (ROS) are formed by glutamate receptor over-activation and inflammatory pathways. It is possible that the ‘retrograde sensitization’ observed by Ferreira and Lorenzetti resulted from the generation of ROS following glutamate and PGE₂ receptor activation. It is well known that SCI generates ROS; thus we hypothesize that ROS contribute to the peripheral sensitization observed following SCI. Of importance to the present consideration of SCI chronic pain, is the role of peripheral sensitization in central neuropathic pain syndromes. While peripheral sensitization has long been suspected to play a role in CNP, we provide the first demonstration of increased responsiveness of peripheral afferents to controlled stimuli after SCI, i.e., peripheral sensitization.

4. Role of Reactive Oxygen Species in Central Sensitization in Regional Neuropathic Pain Following SCI

Reactive Oxygen Species (ROS) in SCI

Immediately after central nervous system injury, extracellular glutamate increases, glutamate receptor mediated intracellular pathways are activated (including increases in intracellular Ca²⁺, COX-2 mediated prostaglandin pathways and the formation ROS, including hydroxyl radicals, superoxide and nitric oxide (Kontos and Wei, 1986; Hall, 2003; Liu et al., 2004)) all of which lead to neuronal and glial death. The resultant oxidative stress promotes neutrophil-mediated inflammation, that further exacerbates secondary damage (Juurlink and Paterson, 1998). Treatments with various superoxide dismutases, enzymes that convert superoxide to hydrogen peroxide, have lowered mortality in rodent models of SCI (Taoka, et al., 1995) and in ischemia or traumatic brain injury in humans (Muizelaar et al., 1993). A novel hypothesis is that ROS contributes to sensitization of dorsal horn neurons (central sensitization) through mechanisms that are currently unknown (see Chung, 2004), but may involve second messenger systems (Kim et al., 2004) and/or glial activation (Raghavendra et al., 2003). We propose that early and chronic removal of ROS after SCI will reduce intracellular signaling pathways that contribute to persistent glutamate receptor activation.

Early sources of ROS after SCI could be contributed by leukocytes (neutrophils, eosinophils, basophils, lymphocytes and monocytes) that are peripherally circulating cellular constituents that invade the spinal parenchyma after SCI (McTigue et al, 2000; Gris et al., 2004) and are thought to contribute to secondary damage through production of ROS (Carlson, et al., 1998). Thus, early treatment with ROS scavengers will provide protection from secondary damage and protect against ROS produced by a variety of cells, both infiltrating and SCI activated astrocytes and microglia (Nesic, et al., 2005). Chronic sources of ROS after SCI are most likely contributed by activated microglia and astrocytes.

Gliopathy Contributes to Chronic Pain

Astrocytes are classically thought to play roles in potassium, glutamate and other transmitter regulation and homeostasis in extracellular and synaptic spaces via uptake mechanisms. Specialized astrocytes also play roles in the blood brain barrier and in neuronal nutritive functions (e.g. satellite cells of dorsal root ganglion neurons). Microglia are classically thought to be principally phagocytes that are mobilized after injury, infection, disease and seizures. When activated, glia cells are known to hypertrophy, increase production of cell specific “markers” (GFAP for astrocytes and OX-42 for microglia and other macrophages) and produce proinflammatory cytokines, ROS, ATP, excitatory amino acids, and nitric oxide (NO) (Johnstone et al., 1999; Martin, 1992; Piani et al., 1992; Shafer and Murphy, 1997; Tanaka et al., 1994); all of which are candidates for mediating pain following neural injury and are known to produce neuronal hyperexcitability in dorsal horn neurons, a necessary substrate for neuropathic pain (Figure 3).

It is interesting to note that the mechanisms of the differential regional pain all appear to have early and permanent activation of both microglia and astrocytes in common (for below-level: Hains and Waxman, 2006, Gwak et al., 2008; for at level-Crown et al., 2008; for above level-Nesic et al., 2005). Perhaps the best of these studies is by Gwak et al., 2008. This study reports that following midthoracic SCI, the application of the phosphodiesterase inhibitor propentofylline (PPF), that modulates both microglial and astrocytic activation (Tawfik, et al., 2007), results in decreased changes in the lumbar cord that include decreased mechanical allodynia, decreased GFAP and OX-42 expression, and decreased glial (both astrocytic and microglia) soma hypertrophy (also a classical marker for activation), as well as decreased neuronal hyperexcitability (Gwak et al., 2008). Thus, the conclusion is that inhibition of glia activation, both astrocytic and microglia, will improve chronic and persistent pain syndromes in remote segments below the level of lesion after SCI and in other central neuropathies. However, it is recognized that the mechanisms of astrocytic and microglial contributions to CNP are likely to be different between the two cell types and are likely to be different regionally in the same cell type (McKay et al., 2007).

In more recent work examining p38 activation, in astrocytes, microglia and dorsal horn neurons just rostral to the level of SCI, Crown et al., 2008 report that inhibiting the enzymatic activity of p38 MAPK reverses mechanical allodynia and decreases hyperexcitability in dorsal horn neurons. In addition, there is a SCI induced increase in GFAP and OX-42 protein expression that is attenuated by blocking activation of p38 MAPK. Thus, the conclusion is that glia activation, both astrocytic and microglia, have important roles in development and maintenance of persistent pain syndromes after SCI and other central neuropathies in regional neuropathic pain syndromes. Moreover, blocking specific intracellular signaling pathways can functionally alter the glia activation response and attenuate neuropathic pain syndromes (Hains and Waxman, 2006, Gwak et al., 2008; for at level-Crown et al., 2008; for above level-Nesic et al., 2005). In the mammalian system, we propose that normal glial function becomes abnormal and dysfunctional after CNS injury. The dysfunctional glial state contributes to conditions that initiate and ensure persistence of neuropathic pain. Whereas the concept of glia-neuronal and neuronal-glia interactions were described in invertebrate systems several decades ago (see Lasek et al., 1974; Villegas, 1972), the conceptual basis of dysfunctional glial cells contributing to neuropathic pain is relatively new (Crown et al., 2008; Detloff, et al., 2008; DeLeo et al., 2006; Gwak et al., 2008; Nesic et al., 2005; Romero-Sandoval, et al., 2008; Milligan et al., 2008).

We propose the term “gliopathy” to describe the dysfunctional and maladaptive response of glial cells to neural injury. We hypothesize that the initiation of gliopathy is the sudden increase in the extracellular concentration of glutamate after peripheral nerve injury (Rooney et al., 2007) and after SCI (McAdoo, et al., 1999) that in some cases is 37 fold higher than resting

concentrations. This results in excitotoxicity (Xu et al., 2008) and glutamate receptor mediated sensitization of both neuronal and glial populations (Hulsebosch, 2005). With respect to the role of glutamate receptors in the dorsal horn excitability, we have published data that all glutamate receptor types (AMPA, kainate, metabotropic and NMDA) are involved in CNP. We know that NMDA receptor activation participates in the upregulation of several proinflammatory molecules (Nesic et al., 2002) and that proinflammatory cytokines exacerbate glutamate-mediated excitotoxicity after SCI (Hermann, et al., 2001). We hypothesize that one of the cellular sources for cytokine production after SCI is from the dysfunctional glial cells. (Of course, infiltrating cells are key contributors of proinflammatory cytokines and other sensitizing agents in early (Fleming et al., 2006) and late SCI (Nesic et al., 2005)). Furthermore, we hypothesize that persistent gliopathy occurs in regions near, as well as, remote from the spinal lesion (including the brain). The dysfunctional glial cells may continue to secrete proinflammatory cytokines and other sensitizing agents in both an autocrine and paracrine manner, creating persistent glial inflammation and continual sensitization of dorsal horn neurons (Hulsebosch, 2008).

Concluding Summary

In summary, it is obvious that the initial glutamate and proinflammatory cytokine increases that occur over the first few hours after SCI are not going to be useful therapeutic targets since most patients present to the emergency rooms 3 hours or later after injury. Additionally, differential regional mechanisms will contribute to the regional chronic pain states. However, we propose the importance of understanding the mechanisms in the differential regional pain syndromes after SCI in the chronic condition. Targeting regional mechanisms will be of enormous benefit to the SCI population that suffer chronic pain, and most likely will contribute to better treatment strategies for other chronic pain syndromes.

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Figure 1.

A massive release of glutamate at the SCI lesion epicenter triggers upregulation of the chemokine CCL21 both at the site of injury and in cell bodies of STT neurons whose axons have been exposed to high concentrations of glutamate. CCL21 is then released at remote sites from the injury activating microglia, that contribute to neuronal hyperexcitability in the spinal cord dorsal horn and thalamus. Abnormal amplification and generation of nociceptive signals at these levels contributes to chronic pain after injury.

Figure 2.

SCI leads to persistent activation of intracellular signaling kinases such as ERK 1/2, p38 mitogen activated protein kinases (MAPK) and calcium calmodulin kinases (CaMKII) that lead to persistent activation of CREB, a transcription factor that contributes directly to factors that phosphorylated NR1 subunits of the NMDA channel, leading to persistent hyperexcitability of spinal and supraspinal neurons. This provides the substrate for persistent or neuropathic pain.

Figure 3.

In the chronic SCI neuron, persistent activation of microglia and astrocytes contribute to production of proinflammatory cytokines, reactive oxygen species (ROS), ATP, excitatory amino acids, nitric oxide (NO) and other factors. Many of these factors, such as specific proinflammatory cytokines (ex. TNF α ; IL-1 α , β) trigger cytokine receptor mediated intracellular pathways in neurons that result in continued activation of NMDA, AMPA and metabotropic glutamate channels as well as cation channels. Thus, neuronal membranes remain hyperexcitable such that incoming sensory subthreshold input (ie. non-noxious stimuli) is now input into a “reset” circuit leading to altered sensory interpretations (ie. non-noxious stimuli becomes noxious: allodynia) or chronic pain.