Implication of Hypothalamus in Alleviating Spinal Cord Injury-Induced Neuropathic Pain

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Key Words

Spinal cord injury · Neuropathic pain · β -Catenin · Hypothalamus · Extracellular signaling regulated kinase pathway · Matrix metalloproteinase

Abstract

Neuropathic pain (NP) is common among spinal cord injury (SCI) patients, and there remain clinical difficulties in treating NP due to the lack of understanding of underlying mechanisms. Extracellular proteins, such as matrix metalloproteinase and β -catenin, have been shown to be activated in the spinal cord regions following an injury, and may play a key role in contributing to NP states. While these extracellular proteins have been used as therapeutic targets in the spinal cord, there has also been evidence of up-regulation in the hypothalamus following a SCI. We hypothesize that the hypothalamus is involved in regulating NP following a SCI, and hence should be researched further to determine if it is a viable target for future therapeutic treatments.

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Introduction

Neuropathic pain (NP) is a significant clinical problem, commonly arising from spinal cord injury (SCI), infectious diseases, and cancer. NP arises directly from damage to the somatosensory system [1, 2]. Primary in-

jury to the somatosensory system is exacerbated by secondary mechanisms such as altered neuron structures, ion channels, inflammation, and potentially aberrant nerve regeneration [2]. An estimated 2.5 million people are affected in various ways by SCI, resulting in significant burden and complications on a patient's lifestyle [3]. SCI commonly results in both motor and sensory dysfunctions. One such sensory disturbance, NP is challenging to manage due to the lack of understanding of underlying mechanisms and limited availability of therapeutic treatments [4]. Numerous extracellular proteins have been studied following a SCI to determine the underlying mechanism of NP. Matrix metalloproteinase (MMPs) and β -catenin are important extracellular proteins associated with immune responses and have been shown to be up-regulated following a SCI. This study focuses on extracellular protein changes associated with NP, and discusses a new hypothesis that the hypothalamus plays a key role in regulating NP activity post SCI (fig. 1). The proposed hypothesis is that the hypothalamus may be a therapeutic target for NP following a SCI.

Implications of MMP and β -Catenin in NP

Extracellular Signaling Regulated Kinase/MMP Pathway

MMPs are key contributors to NP following a SCI [5]. MMPs are zinc-dependent endopeptidases that contrib-

Fig. 1. SCI-induced pain being regulated by the hypothalamus and then relayed to the medulla and spinal cord as discussed in the review.

Fig. 2. Western blot results showing up-regulated MMP-2 in the hypothalamus of male Sprague–Dawley rat model causing pain following a contusion SCI (preliminary data from Dr. Daniel Resnick's lab).

ute to the pathological breakdown and reconstruction of the extracellular matrix (ECM) and extracellular proteins [6]. MMPs play an important role in wound healing and remodeling, however, excessive activation following an injury may contribute to varying pathological conditions, including neuronal cell death, disruption of the blood–spinal cord barrier (BSCB), and ultimately NP [7, 8]. MMPs are able to interact with numerous cell surfaces and soluble proteins including growth factors, adhesion molecules, receptors, cytokines, and chemokines [9]. MMP-9 and MMP-2 are 2 groups of MMPs that play a significant role in secondary physiological recovery following a SCI [10]. MMP-9 and MMP-2 are known to be overexpressed in the acute and chronic phases of NP post SCI [10]. We have demonstrated the up-regulation of MMP-2 in animals in pain (thermal hyperalgesia, TH+) and no pain (TH–) following 21 through 42 days of contusion SCI (fig. 2). MMP-2 and MMP-9 are directly af-

fected through the initiation of extracellular signaling regulated kinase (ERK) pathway. The ERK pathway is associated with the regulation and differentiation of cell survival. ERK pathways are stimulated by neurons damaged in the dorsal root ganglion area, which induce the activation of ERK by protein interactions and phosphorylation in response to signals from nerve cells following a SCI [11]. Phosphorylated extracellular signal-regulated kinase is the activated form of ERK, which causes proliferation and repairing of the cellular matrix [11]. MMP-9 acts as an initiator in the NP cascade by disrupting the BSCB and increasing the number of leukocytes present in the spinal cord [5, 12]. MMP-9 is mainly expressed in glia, macrophages, and neutrophils in the acute phase 24 h following a SCI. MMP-2 is mainly expressed 5 days following a SCI, and is prominent 7–14 days post SCI [13]. MMP-9 has been shown to induce pain through interleukin (IL)-1b cleavage and microglia activation in the early

Fig. 3. Western blot results of β -catenin in the hypothalamus of male Sprague–Dawley rat model showing pain versus no pain expression 42 days following SCI (preliminary data from Dr. Daniel Resnick's lab).

phase, whereas MMP-2 maintains pain through IL-1, and astrocyte activation during the late phase of injury [14, 15].

Employing candidate therapeutic agents such as MMP inhibitors and tissue inhibitors of metalloproteinases (TIMPs) have been shown to inhibit MMP activity, and ultimately alleviate the nociceptive behaviors associated with the activation of MMPs (14). TIMPs have been shown to reduce inflammatory responses, improve locomotive recovery, and decrease tissue damage [10]. Despite evidence of MMP inhibitors alleviating NP, further experiments on MMP activation are necessary to determine a more direct therapeutic target that could eventually be used to alleviate NP in SCI patients. Western blot and immunoprecipitation coupled to gel zymography have revealed MMP expression and activity in the median eminence of the hypothalamus [16]. Evidence of MMP expression in the hypothalamus suggests that the hypothalamus is involved in some aspects of NP, and could be a possible therapeutic target for treating SCI-induced NP in future investigations.

β -Catenin/WNT Pathway

The WNT signaling pathway plays a distinct role in developmental neurogenesis, including regulation of cell migration, induction of MMP expression, and neuronal differentiation [17, 18]. WNTs are a family of lipid-modified signaling proteins that act as both short- and long-term signaling molecules, and bind to Frizzled (Fz) receptors to activate intracellular signaling cascades [19]. The WNT signaling pathway involves β -catenin, a multifunctional protein that interacts with transcription factors to activate the target gene transcription. It also binds to the surface of the ECM to trigger signals involved in the WNT cascade [19, 20]. β -Catenin is expressed in neurons, localized to synaptic contacts, and is one of the synaptogenic factors involved in synapse formation and neural circuit assembly during development [21]. We have observed that β -catenin expression is increased in the hypothala-

mus following SCI in animals with pain (fig. 3). Endothelial cell-derived WNTs, acting through Fz receptors, induce MMP-2 and MMP-9 expression in effector T cells. Expression of the transcription factor β -catenin within the WNT pathway correlates with the increased expression of MMP-2 [22, 23]. In addition, blocking WNT signaling, following induced SCI results in both suppression and persistence of NP [19]. The WNT/ β -catenin pathway also plays a role in the proliferation of hypothalamic progenitors, and should be considered a possible target pathway for NP relief post SCI [18].

Functions of the Hypothalamus and Implications of NP

The hypothalamus functions as a direct connection to the preganglionic neurons of both the sympathetic and parasympathetic nervous systems [24]. Changes to the hypothalamus can cause abnormalities of many homeostatic functions, such as metabolism, thermoregulation, endocrine, or autonomic functions [24]. Metabolic rates and feeding behavior are both regulated by the arcuate nucleus, which converts fuel from sugars and fat during fasting. This is done by working in coordination with the ventromedial and dorsomedial nuclei, the paraventricular nucleus, and the lateral hypothalamus. The regulation of these systems can affect reproduction, thermoregulation, and wake–sleep cycles. Peripheral cold and warm receptors from the spinal cord project to the hypothalamus where thermoregulation is under autonomic control [25]. Damage to the spinal cord can cause disruption between hypothalamic and spinal cord connections, leading to the loss of thermoregulatory functions [25]. Severe SCIs have been reported to cause thermoregulatory abnormalities leading to extreme hypothermia [26].

NP can be triggered from lesions to the somatosensory nervous system [27]. The most common form of pain begins at nerve endings called nociceptors [28]. Regulation

Fig. 4. Alterations in thermal latency times (s) post SCI and before sacrifice were used to divide male Sprague–Dawley rats into pain (P) and no pain groups. These data are significant as seen by the SE bars (preliminary data from Dr. Daniel Resnick's lab).

of nociceptive input originates in the hypothalamus and is relayed to the spinal cord through brain stem nuclei in the medulla [27]. Some chronic pain conditions such as rheumatoid arthritis and fibromyalgia have been directly linked to abnormalities in the hypothalamo–pituitary–adrenal axis (HPA); however, the role of HPA axis on NP is currently unknown [29]. Gene–protein coupled receptors (GPCRs) are the major targets for hormones, neurotransmitters, neuropeptides in the nervous system, and are important for drug development targeting NP [30]. G protein-coupled receptor, family C, group 5 (GPRC5B) have been shown to be related in non-canonical WNT signaling.

Down-regulation of GPRC5B in the dorsal horn of the spinal cord neurons is correlated with an increased NP following a SCI [30]. Not only have GPCRs, such as GPRC5B, been shown as an evidence of NP in the SCI, but GPCRs have also been detected in the paraventricular nucleus and supraoptic nucleus of the hypothalamus [31].

Research into mechanisms behind NP is heavily dependent on animal testing [32]. Therefore, in addition to western blot analysis of MMP and β -catenin expression, pain can be measured based on behavioral tests [32]. Withdraw latency times using a heat stimulus on the hind paws of male Sprague–Dawley rats before and after an induced SCI is a key indicator of NP [32]. Latency times tend to decrease in rats that experience NP following a SCI compared to rats with no NP present, and correlate with injured groups that have up-regulated MMP and β -catenin expression (fig. 4) [32].

Conclusion and Future Studies

Due to the many regulatory functions the hypothalamus has throughout the body, and because of correlations between the hypothalamus and spinal cord expression of main mediating extracellular proteins, we hypothesize that the hypothalamus functions as a regulator of NP following a SCI. The ERK/MMP pathway and the β -catenin/WNT pathway may be considered for future analysis on the hypothalamus to determine the role of the hypothalamus in regulating pain perception. Future studies should be focused on inhibitors of MMP and β -catenin in the hypothalamus to determine if NP states are suppressed. Because TIMPs play a significant role in regulating tissue formation and degradation caused by MMPs, we believe that the effect of TIMPs should be studied in the hypothalamus to determine if the inhibition of MMP activity results in a decrease in NP states. GCPRs in the hypothalamus can also be investigated in future studies as targets for therapeutic agents to determine if abnormalities that arise in the hypothalamus can be treated, in addition to alleviating NP post SCI. While the underlying mechanisms behind NP are complex and mostly unknown, we believe that the hypothalamus should be considered as a target for research into NP following SCI. NP following SCI is a prevalent and devastating complication which currently has no effective treatment. Continued research is required to help alleviate suffering.

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Authors Contribution

A.D.C. wrote this review, including original research by K.K. and G.S.M. from Spinal Cord Injury Laboratory. K.K. received an undergraduate scholarship for the new hypothesis study. K.K. and A.D. induced contusion SCI, harvested tissues following 42 days of injury, performed western blots. G.S.M. planned the experiment design and was also responsible for mentoring. K.K., A.D.C. and A.D. participated in in vivo experiments. D.K.R. directed the study, final review, and editing of manuscript.

Disclosure Statement

The authors have no funding and conflicts of interest to disclose.

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