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Spinal Inhibitory Neurotransmission in Neuropathic Pain

Bradley K. Taylor, PhD

Abstract

Nerve injury increases the spinal cord expression and/or activity of voltage- and ligand-gated ion channels, peptide receptors, and neuro-immune factors that then drive dorsal horn neuron hyperexcitability. The intensity and duration of this central sensitization is determined by the net activity of local excitatory and inhibitory neurotransmitter systems, together with ongoing/evoked primary afferent activity and descending supraspinal control. Spinal endogenous inhibitory systems serve as opposing compensatory influences, and are gaining recognition for their powerful capacity to restrain allodynia and hyperalgesia. These include numerous G-protein coupled receptors (mu and delta opioid, α_2-adrenergic, purinergic A1, neuropeptide Y Y1 and Y2, cannabinoid CB1 and CB2, muscarinic M2, $GABA_B$, metabotropic glutamate type II-III, somatostatin) and perhaps nuclear receptors (PPARγ). Excessive down-regulation or defective compensatory up-regulation of these systems may contribute to the maintenance of neuropathic pain. An increasing number of pharmacotherapeutic strategies for neuropathic pain are emerging that mimic and enhance inhibitory neurotransmission in the dorsal horn.

INTRODUCTION

Peripheral nerve injury produces numerous neurobiological events in the peripheral and central nervous system that contribute to chronic pain. Over the past two decades, with research stemming from the development of new animal models of peripheral nerve injury, we have experienced an explosion of research directed towards an understanding of these pathophysiological neural changes. These include changes in the excitability or activation threshold of primary sensory neurons, increases in descending facilitation or decreases in descending inhibition from the brain, and increases in excitatory signaling or decreases in inhibitory signaling in the dorsal horn of the spinal cord [1,2].

This review begins with an update of the neural mechanisms thought to drive neuropathic pain. It then introduces the growing body of literature devoted to endogenous spinal inhibitory mechanisms, and discusses the current and emerging pharmacotherapeutic approaches that capitalize on the pharmacological plasticity of spinal inhibitory systems after peripheral nerve injury.

NEURAL MECHANISMS THAT DRIVE NEUROPATHIC PAIN

Ongoing Peripheral Nerve Activity

Normally, the spontaneous activity of primary afferent neurons is quite low. In patients with chronic peripheral neuropathy, however, direct microneurographic recordings demonstrated enhanced spontaneous firing of nociceptors innervating the painful region, indicating that abnormally active nociceptors contribute to neuropathic pain. Wall and colleagues found that spontaneous (stimulus-independent) activity and robust mechanical (stimulus-dependent)

Correspondence: Bradley K Taylor University of Kentucky Medical Center 800 Rose Street Department of Physiology Lexington, KY 40536-0298 Tel: 859 323 1870 Fax: 859 323 1070 brad.taylor@uky.edu http://www.mc.uky.edu/physiology/people/taylor.asp.

sensitivity develops in the afferent axonal sprouts innervating the neuroma. The abnormal impulses arising from these sites are called *ectopic* because they do not originate from the normal transduction elements of peripheral terminals of the primary afferent nociceptor[1]. Ectopic activity has been observed in animal models of neuropathic pain, and is thought to arise following either increased expression or redistribution from the soma to the site of injury. The resulting accumulation of sodium channels lowers action potential threshold and causes spontaneous ectopic discharge [1]. Ectopic activity is a key determinant in the generation and maintenance of positive neuropathic symptoms such as spontaneous pain [3].

Phenotypic and structural changes in DRG neurons and their central terminals

Nerve injury elicits a complex pattern of phenotypic changes in DRG neurons, including alterations in the expression of neurotransmitters, neuromodulators, receptors, ion channels, and structural proteins. While some of these changes are related to repair or regeneration, others may contribute to neuropathic pain. Early evidence suggested that nerve injury caused the phenotypic transformation of uninjured Aβ-fibers to a SP-synthesizing mode [4], which were thought to sprout from deeper non-nociceptive regions of the dorsal horn to more superficial nociceptive regions of lamina II [5], where pain transmission neurons are found to express a greater number of NK1 (SP) receptors. Abnormal activation of pain transmission neurons by stimulation of low threshold mechanoreceptors could then produce the touch-evoked pain that is so characteristic of many neuropathies [1]. However, recent rigorous data indicates that such sprouting is very limited [6], and found no evidence that Aβ afferent terminals contain or release SP in the dorsal horn [7]**.

Central sensitization of dorsal horn neurons

Prolonged or massive input from C-nociceptors triggers neuroplastic changes in dorsal horn nociresponsive neurons, leading to their increased spontaneous activity, expansion of receptive fields, and/or decreased threshold to subsequent afferent inputs [1]. This “central sensitization” involves a depolarization-induced influx of calcium through voltage-gated calcium channels, leading to the spinal release of neuropeptides and glutamate from nociceptors, and the subsequent triggering of secondary events such as nitric oxide synthesis, prostaglandin production, protein phosphorylation, and central glial activation. Normally, central sensitization induces an adaptive and protective response that helps to prevent further tissue damage. However, prolonged afferent input, such as sustained ectopic activity after nerve injury, can maintain central sensitization longer than necessary and this could underlie neuropathic pain [3]. In addition to neural mechanisms, the past few years have experienced an explosion of studies investigating the critical contribution of spinal microglia and astrocytes, Schwann cells, satellite cells in the DRG, and immune cells to the central sensitization associated with neuropathic pain. Numerous outstanding reviews have recently been devoted to this topic [8,9].

Supraspinal facilitation of central sensitization in neuropathic pain

Descending projections from the brainstem contribute to the maintenance of neuropathic pain states [2]. For example, the response to noxious mechanical stimulation of pain-facilitatory neurons in the rostral ventral medulla (RVM) increases after nerve injury [10]. Furthermore, selective destruction of facilitatory (mu-opioid receptor-expressing) RVM neurons increased both tactile allodynia and touch-evoked neural activity (Fos expression) within the dorsal horn [11]. Finally, silencing the RVM with local lidocaine injection exerted greater effects on facilitation than inhibition of stimulus-evoked activity of dorsal horn neurons in neuropathic rats, indicating that facilitatory (as opposed to inhibitory) influences from the brainstem predominate during neuropathic pain [12]. Ascending input spinal cord and/or descending

input from cortical and sub-cortical structures can determine the magnitude and duration of descending facilitation, and this remains an important question in need of further research.

Nerve injury-induced loss of intrinsic inhibitory neurotransmission

Central sensitization is not necessarily pathological, and is normally kept in check by a balance of inhibitory controls. However, as discussed next, nerve injury may disrupt one or more endogenous pain inhibitory neurotransmitter systems, thus releasing the brake on central sensitization of dorsal horn neurons and causing stimulus-independent pain.

SPINAL NEUROTRANSMITTER INHIBITION OF NEUROPATHIC PAIN

Traditionally, the majority of pain research laboratories have sought to understand the molecular mechanisms that drive enhanced pain signaling in the dorsal horn, with the long-term objective of developing receptor *antagonists* as new analgesic drugs. These mechanisms include novel gene transcription, post-translational modifications, microglia or astrocyte activation, and alterations in voltage gated ion channels (Nav1.7-1.9, Cav2.2, alpha2-delta subunits), ligand-gated ion channels (neuronal nicotinic, $GABA_A$), ionotropic glutamate receptors (AMPA, NMDA), peptide receptors (NK1), neurotrophins, lipid mediators, and neuro-immune mediators [1,8,13]. By contrast, an increasing number of laboratories have chosen to seek greater understanding of intrinsic pain inhibitory signaling in the spinal cord, with the long-term objective of developing *agonists* at targeted receptors. In addition to traditionally-studied candidates such as opioid, α_2-adrenergic, and $GABA_B$ receptors, more recent attention has been given to alternative G-protein coupled receptors (GPCR), including those for NPY (Y1, Y2), adenosine (A1), cannabinoid (CB1, CB1), acetylcholine (M2), glutamate (groups II and III metabotropic), and somatostatin [14]. The remainder of this reviewer focuses on many of these systems, as well as an emerging non-GPCR target for neuropathic pain, namely, nuclear receptors [15].

Spinal Opioid Inhibition of Neuropathic Pain

Intrathecal morphine produces analgesia in both animals and humans, primarily through naloxone-reversible activation of the μ opioid receptor (MOR). In the dorsal horn, opioid agonists inhibit synaptic transmission at presynaptic terminals by blocking N-type calcium channels, thus reducing transmitter release, and at postsynaptic transmission neurons by opening G protein-coupled inwardly rectifying potassium (GIRK) channels and hyperpolarizing the membrane [14].

Once thought to be resistant to opioids, peripheral neuropathic pain responds moderately well to spinal or systemic opioid therapy [16,17]. However, doses must be pushed into ranges that produce significant side effects, and intrathecal morphine is relatively ineffective in neuropathic pain as compared to transient or inflammatory pain [18]. Recent studies have addressed the spinal mechanisms that underlie this difference. Namely, peripheral nerve section reduces μ opioid receptor (MOR) immunostaining and mRNA expression in injured small DRG neurons and their central terminals [18,19] (but see [20]). As a result, MOR agonists lose their ability to inhibit EPSCs and GIRKs on injured presynaptic terminals and their corresponding superficial dorsal horn neurons, respectively [18,21]. These findings indicate that peripheral nerve injury reduces opioid inhibition of spinal pain transmission from injured Aδ and C fiber nociceptors. This disinhibition could explain loss of opioid sensitivity in patients whose pain is generated by ectopic discharge from injured nociceptors. Indeed, nerve injury-induced loss of spinal MOR is associated with mechanical allodynia in rats [22].

Although the contribution of tonic endogenous opioid tone to transient or acute inflammatory pain is weak at best [23], the opioid receptor antagonist naloxone increases mechanical

allodynia in animal models of neuropathic pain [24]. Furthermore, recent studies indicate that behavioral signs of neuropathic pain are enhanced in μ- or δ-deletion mutant mice [24,25]. Thus, as was originally proposed for clinical dental postoperative pain, opioids appear to orchestrate compensatory neuronal responses that tonically suppress mechanical allodynia in the setting of nerve injury [24,26].

Spinal noradrenergic neurotransmission and neuropathic pain

Spinal delivery of α_2-adrenergic receptor agonists reduces behavioral signs of neuropathic pain in both animals and humans. Antinociceptive mechanisms include α_2-presynaptic inhibition of dorsal root stimulation-induced release of pronociceptive neurotransmitters from primary afferent neurons, and α_1 enhancement of inhibitory synaptic transmission from interneurons in the substantia gelatinosa leading to postsynaptic inhibition of spinal pain transmission neurons [27,28]. Epidural clonidine is approved in the US for the treatment of intractable neuropathic cancer pain, and manipulations that enhance norepinephrine concentrations in the spinal cord (amitriptyline and duloxetine) are first-line treatments for neuropathic pain [17]. Unlike the opioids, however, these compounds decrease neuropathic pain without altering transient nociceptive pain. To explain this, Eisenach and colleagues proposed that nerve injury induces the spinal release of BDNF, which then drives sprouting of terminals from descending noradrenergic fibers [29]**. An intriguing idea is that increased inhibitory NA innervation and release would lead to greater sensitivity to the analgesic effects of duloxetine. In addition, nerve injury increases the efficacy of G-protein coupling to spinal α_2-adrenoceptors, which also could increase the antinociceptive potency of α_2-adrenoceptor agonists [30].

Spinal norepinephrine is derived from the terminals of descending axons originating from noradrenergic brain nuclei, particularly the locus coeruleus (A6), but also A5 and A7 [31]. Further studies are required to determine whether endogenous tone from these supraspinal noradrenergic neurons is part of a tonic inhibitory system that restrains neuropathic pain, or whether they, like opioid receptor-containing neurons in the RVM (above), contribute to descending pain facilitation [32].

Spinal NPY and Neuropathic Pain

Neuropeptide Y and its Y1 and Y2 receptors are highly expressed at key sites of pain transmission, including lamina II of the spinal cord dorsal horn [33,34]. Because NPY inhibits the central release of CGRP from dorsal horn slices [35], these NPY receptors are poised to regulate pronociceptive neurotransmitter release from primary afferent neurons. Furthermore, peripheral nerve injury dramatically increases spinal NPY at the central terminals of low threshold DRG neurons and their central terminals, which appears to be associated with enhanced release in the dorsal horn [36]. As observed with noradrenergic systems, recent studies found that intrathecal administration of NPY decreased behavioral signs of neuropathic pain at doses that do not change transient pain. NPY reduced not only mechanical and cold hypersensitivity, but also spinal Fos expression, a molecular marker of neuronal activation. These anti-allodynic actions of NPY could be reversed either by deletion of the Y1 gene, or by pharmacological blockade of the Y1 or Y2 receptor [37,38]**. The mechanisms underlying the enhanced sensitivity to the analgesic actions of NPY in the setting of peripheral nerve injury remains an important question to address in future studies.

Endogenous NPY also exerts anti-allodynic actions, since germ-line NPY deletion mutant mice display exaggerated autotomy after sciatic nerve transection [39]. (Autotomy is a self-mutilation behavior possibly related to neuropathic pain sensation). Furthermore, unpublished data from our laboratory indicates that conditional NPY knockdown, or pharmacological intrathecal administration of Y2 receptor antagonists, dramatically increases cold and mechanical allodynia after SNI. As noted above for the endogenous opioid system, these

findings suggest that endogenous spinal NPY exerts a compensatory, adaptive inhibition of cold and tactile hypersensitivity in the neuropathic state.

Spinal Adenosine Inhibition of Neuropathic Pain

Intrathecal administration of adenosine reduces allodynia in animals peripheral nerve injury, through both pre- and postsynaptic mechanisms involving Gi-coupled A1-adenosine receptors. At the central terminal of primary afferent neurons, adenosine decreases Ca currents, CGRP release and mEPSP frequency in cultured DRG neurons and/or spinal cord slices [40]. At postsynaptic sites, adenosine hyperpolarizes dorsal horn neurons [41]. Intrathecal adenosine also reduces clinical neuropathic pain, but therapeutic use is limited by major side effects including hyperalgesia [42].

Recent findings indicate that thiamine monophosphatase (TMP, also known as fluoride-resistant acid phosphatase, the transmembrane form of prostatic acid phosphatase), a classic marker of small-diameter DRG neurons and their central terminal in the dorsal horn whose expression plummets after nerve injury, is one of the ecto-5'-nucleotidase enzymes that can rapidly dephosphorylate AMP to adenosine [43]**. Intrathecal injection of TMP decreased behavioral signs of neuropathic pain in normal mice, but neither in A1R deletion mutant mice nor in the presence of an A1R antagonist [43]. These innovate results identify a new approach for pharmacological treatment of neuropathic pain: a treatment that selectively increases spinal TMP activity should generate inhibitory A1-adenosinergic tone that dampens the severity of neuropathic pain.

Cannabinoid receptor agonists and neuropathic pain

Several clinical studies indicate that cannabinoids such as delta(9)-tetrahydrocannabinol (THC) or Sativex (THC plus cannabidiol), reduce neuropathic pain, but these compounds also produce adverse effects such dizziness and sedation at therapeutic concentrations [44]. Two intriguing approaches based on recent innovative basic science studies are currently under investigation to overcome these limitations.

First, selective targeting of peripheral CB1 receptors may provide significant pain relief with fewer CNS side effects. Conditional deletion of CB1 on sensory neurons increased behavioral signs of neuropathic pain and decreased the antinociceptive effects of the CB1 agonist, WIN 55212-2 [45]**. These data indicate that CB1 expressed by nociceptor neurons, rather than CNS neurons, primarily mediates cannabinoid-induced inhibition of neuropathic pain. Peripherally-acting CB1 agonists are in development [13].

Second, spinal administration of the CB2 receptor agonist JWH-133 attenuated mechanical allodynia in neuropathic rats, an anti-allodynic that could be reversed with the CB2 antagonist SR144528 [46]*. Because CB2 is not normally expressed in the CNS, these results were initially puzzling, until it was discovered that nerve injury induces the *de novo* expression of CB2 on activated microglia [47]. Subsequently it was revealed that intrathecal JWH-133 reduced microglial activation as well as nerve injury-induced allodynia [46]. In summary, sensory neuron CB1 and spinal cord glial CB2 receptors represent important emerging targets for the treatment of neuropathic pain.

Spinal GABA and modulation of neuropathic pain

Interneurons containing γ-aminobutyric acid (GABA) or glycine inhibit the spinal transmission of noxious sensory signals, and numerous studies indicate that spinal GABAergic inhibition is reduced after experimental nerve injury [48]. How this occurs has become quite controversial in recent years, in some cases with the presentation of strong data that counter prevailing theory. First, caspase-3 and TUNEL were initially reported in the dorsal horn after nerve injury,

indicating death of inhibitory neurons [48,49]. However, Polgar and Todd conducted rigorous anatomical studies indicating that loss of neurons in lamina I-III is minimal and is not required for the development of tactile allodynia, and noted that TUNEL positive nuclei belonged to microglia not neurons [50]. Second, spinal GABA and GABA immunoreactivity was initially found to decrease in the dorsal horn after nerve injury reduced [51], but this could not be confirmed with rigorous light and electron microscopic analyses [52]**. Third, nerve injury does not decrease $GABA_A$ receptor immunoreactivity [48,52]. Fourth, De Koninck and colleagues have suggested that nerve injury reverses the chloride concentration gradient in lamina I, effectively converting GABA from an inhibitory to an excitatory neurotransmitter [53]. However, this mechanism implies that the overall effect of GABA is pain facilitatory, which is difficult to reconcile with numerous reports that $GABA_A$ and $GABA_B$ receptor agonists reduce allodynia and hyperalgesia [54]. Fifth, diminished primary afferent nociceptor activity following nerve injury might reduce the excitability or burst firing patterns of GABAergic interneurons; however, recordings from spinal cord slices with roots attached indicate that input from Aδ- and C-fibers do not differ between neuropathic and sham-operated rats (input from Aβ-fibers could not be tested in this study) [55]. In order to capitalize on robust inhibitory GABAergic neurotransmitter in the dorsal horn, further studies are needed to determine the mechanisms underlying decreased GABAergic tone after nerve injury and its relationship to neuropathic pain.

Spinal muscarinic inhibition of neuropathic pain

Intrathecal or systemic administration of cholinesterase inhibitors produces analgesia in animals and humans, likely via M2 muscarinic receptors in the dorsal horn [14,56]. Nerve injury increases the expression of M2 cholinergic receptors on sensory afferent [57], suggesting that cholinergic inhibitory tone is augmented after peripheral nerve injury.

Spinal Nuclear Receptors and Inhibition of Neuropathic Pain

The gamma subtype of peroxisome proliferator-activated receptor (PPARγ), a nuclear receptor, was recently found to be expressed in the spinal cord [15]**. Activation of these receptors inhibits allodynia, since intrathecal administration of PPARγ ligands dose-dependently attenuated nerve injury-induced mechanical and thermal hypersensitivity in a PPARγ antagonist-reversible manner [15]. These data suggest that new or currently available drugs targeted at spinal PPAR-gamma (such as pioglitazone, which is approved for the treatment of diabetes by the United States Food and Drug Administration) may yield important therapeutic effects for the management of neuropathic pain.

Furthermore, a PPARγ antagonist itself rapidly increased the mechanical allodynia associated with nerve injury [58]**. Although additional studies are required to identify the molecules that intrinsically activate PPARγ in the dorsal horn, these studies suggest that endogenous PPARγ tone dampens the severity of neuropathic pain.

CONCLUSIONS

The past few years have experienced a generational shift in our efforts to understand the spinal mechanisms of neuropathic pain. As summarized in Table 1, trends in research focus include: 1) a shift from phenotypic and structural changes that drive pain to mechanisms of pharmacological plasticity that strengthen analgesia; 2) a shift from a few studies on neuron-immune interactions to an explosion of work on the reciprocal connections between neurons, glia, and immune cells in the dorsal horn that cause hypersensitivity; 3) a shift from loss of spinal GABAergic inhibition to reversal of $GABA_A$-associated chloride channels; 4) a shift from an emphasis on GABA-, α_2 receptor-, and opioid-mediated inhibition to a broader study of numerous other spinal inhibitory systems including cannabinoid, adenosine, NPY, and

PPARγ; and 5) increasing recognition that the central sensitization that underlies neuropathic pain is opposed by compensatory influences involving the up-regulation of spinal inhibitory systems. Normally, such systems maintain a powerful capacity to restrain allodynia and hyperalgesia. Figure 1 proposes that if nerve injury does not induce these normal feedback inhibitory mechanisms, then central sensitization in the dorsal horn will remain unchecked, leading to neuropathic pain.

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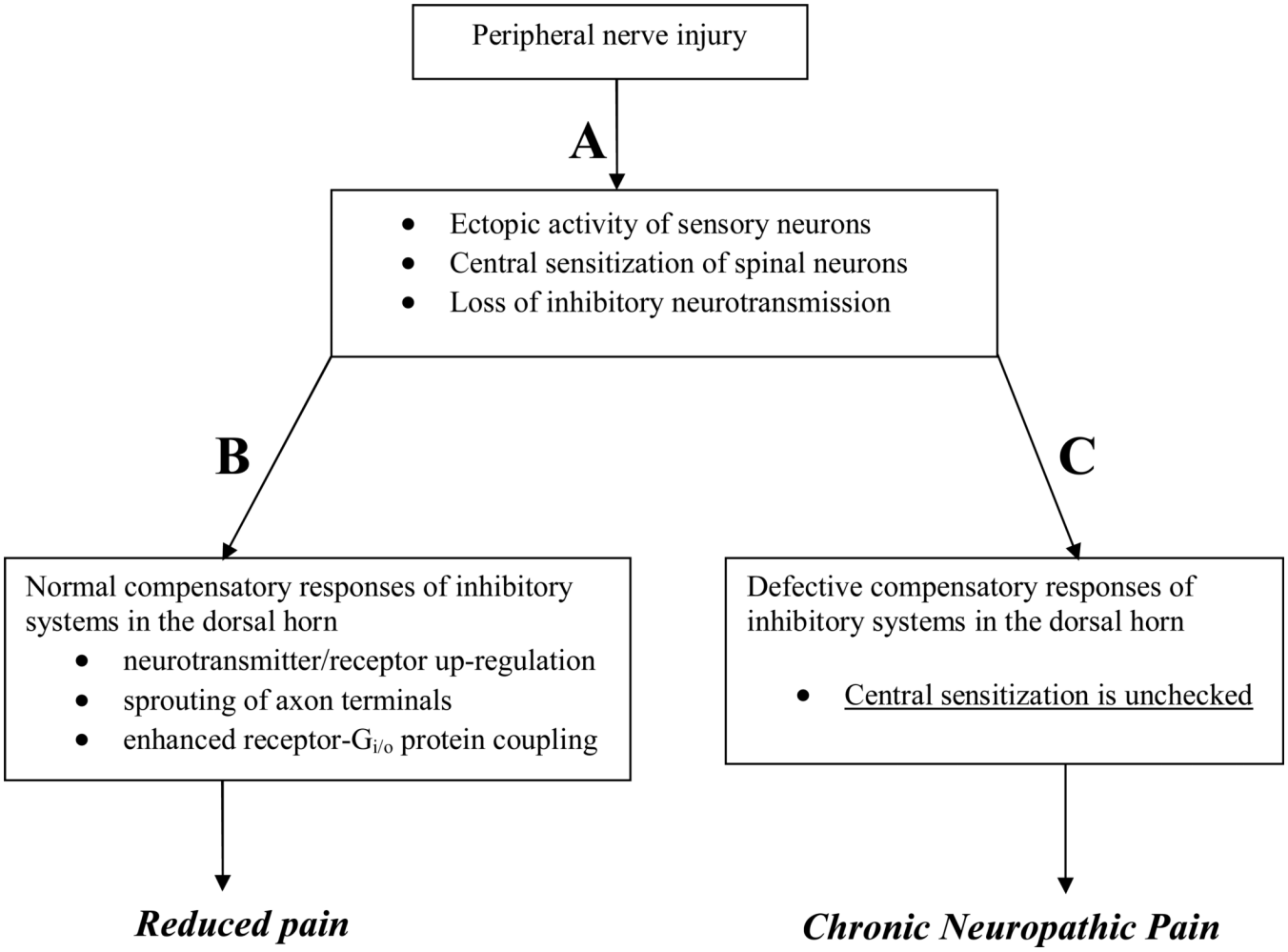

Figure 1.
Proposed sequence of events leading to: **A)** Early spinal nociceptive transmission and pain and; **B)** normal resolution of pain following compensatory up-regulation of inhibitory system;, or **C)** pathological development of neuropathic pain when compensatory mechanisms fail.

Table 1

Modification of endogenous inhibitory mechanisms in the spinal cord after nerve injury

NPP = Neuropathic Pain. TMP = thiamine monophosphatase, which generates the inhibitory neurotransmitter, adenosine. Receptor down-regulation could induce loss of opioid sensitivity. Conversely, noradrenergic sprouting, enhanced α_2 receptor-G protein coupling, and de novo CB2 expression could explain the enhanced efficacy of duloxetine, clonidine, and cannabinoids as analgesics for neuropathic pain, respectively.

Mechanism	Recent Example	Proposed Impact on NPP
Receptor down-regulation	Mu-opioid	unimpeded
Enzyme down-regulation	TMP [43]	unimpeded
Reversal of chloride channel	$GABA_A$	unimpeded
Sprouting of nerve endings	noradrenergic [29]	constrained
Receptor-G protein coupling	α_2 receptor [30]	constrained
de novo expression in microglia	CB2 receptor	constrained
Neurotransmitter up-regulation	Neuropeptide Y	constrained
Receptor up-regulation	M2 receptors	constrained