

Spinal and Supraspinal Contributions to Central Sensitization in Peripheral Neuropathy

Rie Suzuki · Anthony Dickenson

Department of Pharmacology, University College London, London, UK

Key Words

5HT · 5HT3 · Spinal cord · Lamina I · Gabapentin · Descending excitation · NK1 receptor · Substance P

Abstract

We will focus on spinal cord dorsal horn lamina I projection neurones, their supraspinal targets and involvement in pain processing. These spinal cord neurons respond to tonic peripheral inputs by wind-up and other intrinsic mechanisms that cause central hyper-excitability, which in turn can further enhance afferent inputs. We describe here another hierarchy of excitation – as inputs arrive in lamina I, neurones rapidly inform the parabrachial area (PBA) and periaqueductal grey (PAG), areas associated with the affective and autonomic responses to pain. In addition, PBA can connect to areas of the brainstem that send descending projections down to the spinal cord – establishing a loop. The serotonin receptor, 5HT3, in the spinal cord mediates excitatory descending inputs from the brainstem. These descending excitatory inputs are needed for the full coding of polymodal peripheral inputs from spinal neurons and are enhanced after nerve injury. Furthermore, activity in this serotonergic system can determine the actions of gabapentin (GBP) that is widely used in the treatment of neuropathic pain. Thus, a hierarchy of separate, but interacting excitatory systems exist at peripheral, spinal and supraspinal sites that all con-

verge on spinal neurones. The reciprocal relations between pain, fear, anxiety and autonomic responses are likely to be subserved by these spinal-brainstem-spinal pathways we describe here. Understanding these pain pathways is a first step toward elucidating the complex links between pain and emotions.

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Introduction

Pain research is an advancing field of neuroscience asking questions about pain perception and basic neurobiological transmission in terms of how the central nervous system (CNS) processes sensory information. In terms of medical and social issues, a yet more important aspect of this research is knowledge that could improve the treatment of pain, particularly chronic pain. A fundamental approach to understand pain processing is to define the neuronal networks that mediate and modulate sensory inputs, and how trauma and disease may alter their function. Noxious stimulation evokes changes in many systems within the CNS; different chronic pain states generate unique pharmacological patterns in the brain. Models of pain in integrated systems are essential to this goal and our main hypothesis is that different pain states evoke different changes. New insights into how sensory information is centrally processed are fundamental

to better pain control and development of new analgesics.

As pain persists there are the affective and emotional responses that must be considered along with the sensory aspects of the stimulus. It is clear that although the sensory and psychological aspects of pain are separable, the neural pathways that contribute to these aspects of pain are inter-linked. Furthermore, at both peripheral and central sites, there are mechanisms that can amplify and prolong the painful stimulus so that the pain becomes greater – this can result in severe pain in the presence of relatively minor peripheral pathology. There is considerable capacity for change in both the transmission and modulating systems in these prolonged pain states, and plasticity occurs at both peripheral and central sites. Recently, a lot of attention has been given to the expression of genes in chronic pain models [1] – this may well pay dividends in terms of novel targets; however, it is equally likely that enhanced activity of constitutive systems could activate novel signalling pathways to alter pain levels markedly without the production of new proteins. This review will discuss changes in the central processing of noxious inputs that we believe to have marked and selective influences on both the level of pain signalling and the actions of therapeutic agents.

Pain starts in the periphery; acute pain causes the activation of peripheral C-fibres by activating nociceptors that respond to noxious mechanical, chemical or thermal stimuli. Peripheral events that accompany nerve injury are quite different from peripheral sensitization seen in inflammation, and it is paradoxical that damage to peripheral nerves not only give rise to expected negative symptoms (sensory deficits) but a proportion of patients report positive phenomena (abnormal spontaneous or evoked sensations). There is substantial evidence to suggest that alterations in somatosensory processing following nerve injury originate in the peripheral nervous system and these have been reviewed extensively [2]. Neuropathic pain, however, can be explained only partly by peripheral changes because patients can have severe pain yet also marked sensory loss – thus compensations within the central nervous system appear to be of major importance [3].

Central Spinal Excitatory Events

The first synapse in the transmission of noxious information from the periphery to the brain is in the superficial dorsal horn of the spinal cord, which is comprised of lam-

ina I and II (the marginal zone and substantia gelatinosa, respectively) [4]. Lamina I of the spinal cord plays a key role in the modulation of pain transmission and these neurons have distinct response properties compared to deeper lying neurones (lamina V). Electrophysiological recordings of lamina I neurons show that the majority of these neurons (around 75%) are nociceptive-specific (NS) in their responses, about 15% are termed polymodal nociceptive (HPC) with an additional response to noxious cold, and the remainder (10%) are wide dynamic range (WDR). In lamina V the large majority are WDR so that information transmitted from these two major groups of dorsal horn neurons are almost entirely nociceptive in lamina I while lamina V neurons code in the innocuous through the noxious range. Lamina I neurons exhibit higher thresholds for excitation and generally have smaller mechanical and heat-evoked responses and receptive fields when compared to deeper dorsal horn neurons.

Superficial dorsal horn neurons are morphologically diverse. Lamina I neurons typically have long axons thus allowing projection to higher CNS centers [5]. This is not the case for lamina II, which is comprised mainly of small intrinsic neurons terminating locally [6]. A small proportion of lamina I neurons appears to project contralaterally via the lateral spinal thalamic tract (STT), which is a key pathway for pain, itch and temperature [7, 8], however the predominant ascending output from lamina I neurons appears to be the spino-parabrachial pathway in the rat [5, 9, 10]. Neurons in the deep dorsal horn (lamina V–VI), however, have predominant projections in the STT. The spinothalamic pathway is important for the sensory discriminatory aspects of nociception, whilst the spino-parabrachial pathway plays a key role in affective reactions to pain mechanisms and in regulating descending control systems [11].

Neurons in the parabrachial area (PBA) show specific activation by noxious stimuli and generally have large receptive fields covering a number of body parts [12]. This lack of somatotopy suggests roles in diffuse responses to pain and damage, rather than fine and localized information [13]. The two main forebrain targets of parabrachial neurons are the nucleus centralis of the amygdala and the ventrolateral medial hypothalamus [14]. Projections to the amygdala probably contribute to some components of aversive *emotions* such as fear and anxiety, whilst the PBA-hypothalamus-PAG circuits may be involved in aversive behaviors such as rage and aggression [12]. The PAG is closely associated with areas of the brainstem including the rostral ventromedial medulla (RVM), and is critical in the descending modulation of

spinal activity through monoaminergic and other pathways [15,16]. The PAG and RVM can exert both facilitatory and inhibitory influences on the spinal cord [17, 18]. That these influences can be modulated by different manipulations suggests they involve anatomically distinct pathways [19]. The net balance of these two supraspinal pathways, together with primary afferent input, will ultimately determine the excitability of spinal neurons. Thus, the PBA receives specific noxious inputs from numerous spinal neurons then relays to brainstem sites that reciprocally project back to the spinal cord. These interacting pathways will form complex loops so the brain is first informed of spinal nociceptive activity but further regulation of spinal responses could be produced by the descending pathways [16].

The arrival of sensory information from nociceptors into the dorsal horn considerably alters the level of activity within the cord since both excitatory and inhibitory systems can impinge upon spinal neuronal activity. These influences can originate both intrinsically and supraspinally. Calcium channels on afferent terminals and neurons are important for both transmitter release and determining neuronal excitability. Augmented release of afferent transmitters, including peptides such as calcitonin gene-related peptide (CGRP) or substance P (SP), together with glutamate allows changes in their postsynaptic receptor actions. One of the key events is the activation of the N-methyl-D-aspartate (NMDA) receptor for glutamate that underlies wind-up, whereby the baseline response to a constant noxious input is amplified and prolonged [20, 21]. This forms the basis of central hypersensitivity which results in an increased responsiveness of dorsal horn neurons, often observed in persistent inflammatory and neuropathic pain states. Recent data point to yet another type of facilitatory drive from within the brainstem (in particular, the RVM) that can enhance spinal mechanisms of pain, independently in some cases or alongside those mentioned previously. There is accumulating evidence for the contribution of these brainstem excitatory influences in the development and maintenance of central sensitization in persistent pain states (for review, see [17]). Here we emphasize systems that arise from lamina I neurons of the spinal dorsal horn that link to descending pathways from the brainstem. We will consider the relation between sensory inputs and emotional areas of the brain and the mechanisms by which central pharmacological systems can enhance pain.

Lamina I Pathways and Descending Facilitation

We have recently shown that selective ablation of lamina I NK1 receptor expressing neurons through the use of a SP-cytotoxin conjugate (SP-SAP) [22] produces profound effects on the response characteristics of deep dorsal horn neurons [23]. Approximately 45% of neurons in lamina I appear to express NK1 receptors [24] and ablation of these neurons resulted in (a) a reduction in receptive field size; (b) disruption in mechanical and thermal coding; (c) reduced responses to chemical inflammation, and (d) reduced central sensitization of deep dorsal horn neurons as revealed by diminished wind-up. These lamina I neurons therefore link peripheral afferent stimuli to the sensory coding of deep WDR neurons, and provide a major input to the brain [23]. As these cells form part of an important ascending pathway to the brainstem, one reason for the reduced responsiveness observed in deep dorsal horn neurons following SP-SAP treatment is likely to be due to the loss of the lamina I-PBA pathway. The latter may form part of a loop back to the spinal cord, and appears to be supported by pharmacological evidence. The anatomical and pharmacological evidence for descending pain facilitatory pathways is mounting; activation of the excitatory 5HT₃ receptors in the spinal cord by serotonin released from pathways projecting from the RVM has been suggested to underlie the descending excitatory drive from the RVM to the spinal cord [23]. The 5HT₃ receptors are predominantly localized in the superficial dorsal horn [25, 26] on nerve terminals of small diameter afferents [27]. Blocking spinal 5HT₃ receptors using the selective antagonist ondansetron (Zofran™) and related drugs has implicated a pronociceptive role of these receptors [28, 29]. Importantly, most of the effects of ablating these lamina I neurones were reproduced by blocking descending serotonergic facilitatory pathways using spinally administered ondansetron in unlesioned animals. Thus, NK1-lamina I projection neurons forms the origin of a spino-bulbo-spinal loop, and consequently control spinal excitability at least in part through the activation of a descending serotonergic pathway.

It has to be pointed out that analgesic effects of activation of this receptor have also been reported by some studies. The apparent discrepancies may be due to variable test doses of drugs, assays, species differences, or the possibility that the excitatory action of 5HT₃ may induce diffuse noxious inhibitory controls (DNIC); excessive excitation could also trigger depolarizing block of neuronal activity. Recent data from 5HT₃ knock-out mouse sup-

port the pronociceptive role of this receptor in the spinal cord [23]. Only wind-up did not depend on this 5HT₃ receptor-mediated pathway, thus confirming the importance of spinal circuits for this neuronal measure [23]. In fact, it is clear from the variety of preparations that have been used to study wind-up that this short term increase in excitability is an intrinsic spinal event seen in spinalized animals, slices and neuronal co-cultures [21]. This leads to the intriguing possibility that quite different messages may be sent to the emotional/autonomic areas of the brain compared to the sensory-discriminative zones. Neurons in lamina I of the spinal cord have projections to PBA/PAG whereas many deep cells project in the STT tract. NMDA-dependent wind-up is clearly seen in the large majority of deep dorsal horn cells yet is almost absent in lamina I cells. This indicates that spinothalamic inputs will be potentiated through spinal wind-up-like mechanisms, whereas afferent messages to PBA will not. This may consequently lead to dissociation between the emotional and sensory-discriminative aspects of pain. However, both neuronal types support LTP when high-frequency stimuli are given [30–32].

Central Pain Pathways and Neuropathic Pain

As already discussed, neuropathic pain is driven by multiple peripheral and spinal mechanisms [33–35]. In addition to these alterations, nerve injury leads to enhanced descending excitatory drive from the RVM to maintain the chronic pain states [36]. Disruption of ascending or descending pathways effectively blocks abnormal pain after neuropathy, supporting the idea that these descending facilitatory systems contribute to the development of pain that follows peripheral nerve injury [37]. Cells of the RVM fall into three types, one of which has been associated with pronociception, and these neurones have been proposed to be the source of descending facilitation from the RVM [38]. Hence one possibility for the abnormal pain associated with neuropathic pain is an inappropriate activation of these brainstem cells which would promote excitatory influences onto the spinal cord and facilitate nociception [37]. This may represent a central compensatory mechanism for the loss of normal sensory input that results as a consequence of peripheral nerve damage [34]. Under pathological conditions descending facilitatory controls to the spinal cord are likely to be enhanced and excitatory influences predominate to maintain spinal central sensitization. Given that the brainstem areas involved in these sensory controls are

also implicated in emotions, sleep and autonomic responses, the well established links between these states and pain may well involve these same pathways and provide a basis for alterations in pain as a result of affective changes [39].

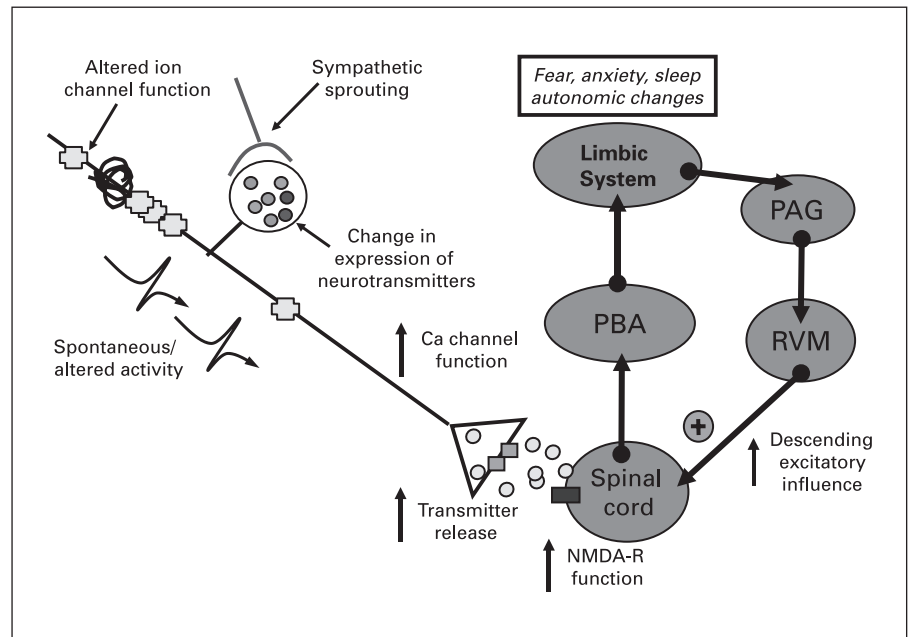
Both chemical and electrical stimulation could evoke facilitations at doses and currents less than those required to elicit inhibitions (see refs in [17]). Thus, the balance between serotonergic and non-serotonergic excitatory and inhibitory controls may be critical for determining the level of spinal activity.

5HT in Central Pain Pathways

Currently, the main focus of 5HT₃ receptor antagonists such as ondansetron has been on the clinical treatment of postoperative and chemotherapy-induced emesis, and only limited evidence exists for the use of this class of drugs in pain management [40]. Our recent findings of an increased efficacy of ondansetron on mechanical punctate evoked responses following peripheral nerve injury favors the potential clinical use of this agent for the treatment of neuropathy, particularly in patients with tactile allodynia. A preliminary clinical study suggests that block of 5HT₃ receptors does indeed have clinical utility in the treatment of pain (fig. 1) [59].

More recently, we observed that ondansetron exerts greater effectiveness after nerve injury compared to sham controls, particularly on mechanical punctate responses [60]. This was clearly shown by the robust inhibition of these responses following the administration of a low dose of ondansetron, which under normal conditions, produces little or no effect in control animals. Our results support the idea that supraspinal serotonergic inputs to the spinal cord may have sustained facilitatory influences to the spinal cord following injury to peripheral nerves. Although there is a downregulation in the 5HT₃ receptor gene in injured L5 and L6 DRG in the spinal nerve ligation (SNL) injury model, the gene is not altered in the neighboring uninjured L4 [1] suggesting that this receptor could function to alter evoked pain in this model. An interesting finding was the observation that ondansetron produces more selective and robust actions on mechanical punctate inputs compared to thermal inputs, thus leaving the heat responses relatively intact. The 5HT₃ receptor has been localized to a novel group of small diameter afferents that are not IB4 positive, non-peptidergic, contain very few vanilloid receptors (TRPV1), and a larger number of presumed A-delta afferent fibers [27]. In vitro, 16% of A-

Fig. 1. Peripheral nerve injury induces changes in both the peripheral and central nervous system. Spontaneous activity is generated at the site of injury and the DRG. Plasticity is seen in the expression and function of ion channels (e.g. Na⁺ channels) and neurotransmitters (e.g. SP). Sprouting of sympathetic nerve fibers in the DRG act to sensitize peripheral afferents. increased Ca²⁺ channel function will result in enhanced neurotransmission thereby promoting sensory transmission in the spinal cord. Centrally, there is increased function of the NMDA receptor and enhanced activity in serotonergic pathways arising from the brainstem. All these mechanisms can contribute to the development of abnormal pain accompanying nerve injury.



delta fibers are activated strongly by a 5HT₃ agonist and a larger proportion of C-fibres (29%) respond albeit with lower levels of firing [27]. In keeping with this characteristic, a recent study on sensory afferents from the colon showed that the majority were high-threshold afferents and 30% responded to a selective 5HT₃ agonist, 2-methyl-5HT [41]. 26% of cell bodies retrogradely labelled from the colon displayed 5HT₃ receptor-like immunoreactivity. The finding that the receptor is located on a subgroup of afferents suggests that the descending facilitations that act through this receptor would have selective effects. Moreover, the selectivity over mechanical vs. thermal inputs implicates a pre-synaptic mechanism since post-synaptic effects are likely to alter all activity. This pattern of effect agrees with what we have observed with SP-SAP and also fits well with behavioral data showing spinal section has a greater effect on mechanical allodynia than thermal responses in animals with nerve injury [42]. Furthermore, additional actions of 5HT may be mediated through post-synaptic and intrinsic systems in the dorsal horn [25, 26]. Enkephalin-immunoreactive neurons in the superficial dorsal horn are innervated by 5-HT-immunoreactive fibers. Using single-cell RT-PCR 5HT₃ receptor mRNA was detected in ~25% of enkephalinergic neurons. Although some of the antinociception elicited by activation of 5HT₃ receptors at the spinal cord may involve enkephalinergic dorsal horn neurons, this cannot explain the pronociceptive effects of this receptor.

The 5HT₃ receptor has also been shown, by merit of its presynaptic location, to open calcium channels, causing long slow openings of these channels that would lead to increased transmitter release. Interestingly, presynaptic 5HT₃ receptors are found throughout the brain, with differences in the kinetics of their responses in different brain regions [43]. Only a proportion of the synaptosomal population responded to 5HT₃ agonists, suggesting that these receptors are able to regulate the functioning of select synapses. Afferent activity may also regulate the 5HT₃ receptor since in vitro electrophysiological recordings revealed that calcium influx increases the cytoplasmic Ca²⁺ concentration and markedly reduces desensitization of the 5HT₃ receptor channel [44]. Thus, there is cross-talk between voltage-gated Ca²⁺ channels and 5HT₃ receptors. We therefore hypothesized that one consequence of activation of this serotonergic system would be an interaction with drugs that act to alter transmitter release. One candidate would be gabapentin (GBP), which is an anticonvulsant widely used for the treatment of various types of neuropathic pain [45–48]. The mechanism(s) of its anticonvulsant/analgesic actions remain to be determined. GBP binds to a unique site in the CNS identified as $\alpha_2\delta$ which is a modulatory accessory subunit of the voltage-dependent calcium channels (VDCC) [49, 50], and may regulate the function of VDCCs. Despite the fact that the $\alpha_2\delta$ subunit is common to all VDCCs, GBP is clinically efficacious against chronic pain with minimal

adverse effects [51]. Behavioral studies show GBP is ineffective against physiological sensory nociception but produces robust antinociception following pathology such as nerve injury and inflammation [51–57]. In all of these studies GBP was effective against mechanical inputs; however, for thermal responses, mixed effects have been reported with the majority of positive effects being seen in inflammatory rather than neuropathic models.

We administered spinal ondansetron at an effective dose in the SNL induced neuropathic rats and observed the expected inhibition of evoked responses of the dorsal horn neurons. However the administration of a moderate dose of GBP, during the inhibition produced by block of the 5HT₃-mediated facilitation, was now entirely without effect. Thus blockade of the 5HT₃ receptor prevented GBP from inhibiting neuronal activity, suggesting that both agents may have similar pre-synaptic action. The depolarizing effects of 5HT₃ receptor activation on afferent terminals may thus prolong calcium channel opening. This may then allow the interaction of GBP with the $\alpha_2\delta$ subunit to reduce VDCC activity and inhibit transmitter release. One functional commonality between the two drugs is their greater effectiveness on mechanical, rather than thermal activity, which fits with the lack of colocalization of the 5HT₃ receptor and VR-1 on afferent fibers [27]. Furthermore, ablation of the NK-1 expressing neurones, ondansetron and GBP administration, and genetic deletion of 5HT₃ receptor lead to very similar effects on the formalin response. It is unlikely that post-synaptic actions of any drug could underlie these selective effects

on dorsal horn neuronal responses as the majority of both superficial and deep dorsal horn neurones respond to mechanical, thermal and chemical stimuli. However, one study did report differential activation of defined populations of dorsal horn neurons, suggestive of a separation of mechanical and thermal inputs. In lamina II of the dorsal horn, 15% of a population of neurons expressing the MOR1 receptor responded (c-fos) to thermal stimulation but failed to respond to formalin or mechanical stimulation [58]. We propose that the interactions between the 5HT₃ receptor and calcium channel modulation by GBP at presynaptic sites allow preferential actions on mechanical (and chemical)-evoked responses.

Conclusions

The anatomy and pharmacology of descending facilitation is not yet fully understood, nor the extent to which this mechanism underpins chronic pain states arising from peripheral neuropathy. Nonetheless, what is clear is that greater understanding and elucidation of pain pathways, their receptors and substrates, can pave the way towards improved pain therapy.

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