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Contribution of the Spared Primary Afferent Neurons to the Pathomechanisms of Neuropathic Pain

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Abstract

Neuropathic pain is caused by nervous-system lesions. Early studies on the pathomechanisms of this abnormal pain state have focused on the directly injured fibers and neurons. Here, we present recently accumulating data about the contribution of the primary afferent neurons spared from direct injury to the pathomechanisms of neuropathic pain. The phenotypic changes in the spared neurons are similar to those in the neurons in peripheral inflammation models, as opposed to those in the directly injured neurons. Electrophysiological changes and behavioral data also favor the contribution of the spared neurons. These attractive targets of study will give us new approaches for understanding the abnormal pain.

Index Entries: neuropathic pain; spared DRG neurons; phenotypic change; substance P; calcitonin gene-related peptide; brain derived neurotrophic factor; vanilloid receptor subtype 1; $P2X_3$; spontaneous discharge; behavior.

Introduction

Primary afferent neurons, existing in trigeminal and dorsal root ganglia (DRG), play roles in the early stage of sensory processing: transduction of environmental stimuli to electrical activity at the peripheral terminals, conduction of the activity through their axons to the spinal dorsal horn, and synaptic transmission at the central terminals. For these roles, primary afferent neurons possess many molecules including receptors, ion channels, transmitters, and neuropeptides. In some pathological conditions, primary afferent neurons change the expression of some of these molecules, and these phenotypic changes have been thought to contribute to the pathomechanisms of abnormal persistent pain.

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Phenotypic Change in Peripheral Inflammation Models

Inflammation in peripheral tissue is a common pathological state that produces pain. The pathomechanisms of inflammatory pain have been studied using animal models of acute and chronic inflammation produced by intradermal injection of chemicals, such as carageenan and complete Freund's adjuvant. In general, inflammatory pain is caused by activation and/or sensitization of the primary afferents by inflammatory mediators, such as histamine, bradykinin, 5-hydroxytriptamine, ATP, and protons, which are derived from the damaged tissues and inflammatory cells. In addition, it has been demonstrated that excitatory neuromodulators, such as substance P and brain-derived neurotrophic factor (BDNF), increase in the primary afferent neurons and are likely involved in the pathomechanisms of prolonged inflammatory pain *(1,2).*

Phenotypic Change in Neuropathic Pain Models

Neuropathic pain is an abnormal persistent pain caused by injury to nerves. Widely used animal models involve partial injury of the sciatic nerve or its components. Therefore, directly injured and spared primary afferent neurons exist in the corresponding DRG. The phenotypic changes of the directly axotomized DRG neurons have been widely studied, some of which should contribute to the pathomechanisms of neuropathic pain. In this review, we focus on the recent articles on changes in uninjured DRG neurons that had been ignored.

In addition to the traditional three models; chronic constriction injury (CCI) of the sciatic nerve *(3),* partial sciatic nerve ligation (PSNL) *(4),* and L5 (and L6) spinal nerve ligation (SPNL) *(5),* a new model named "spared nerve injury (SNI)" has recently been proposed *(6)* (Fig. 1). All of these models cause spontaneous pain behavior, exaggerated response to noxious stimuli (hyperalgesia), and/or abnormal pain to innocuous stimulation (allodynia) of the plantar surface of the hindpaw. Because the plantar surface of the rat hindpaw is innervated by the L3-5 spinal nerves *(7),* and the sciatic nerve is mainly composed by L4-6 spinal

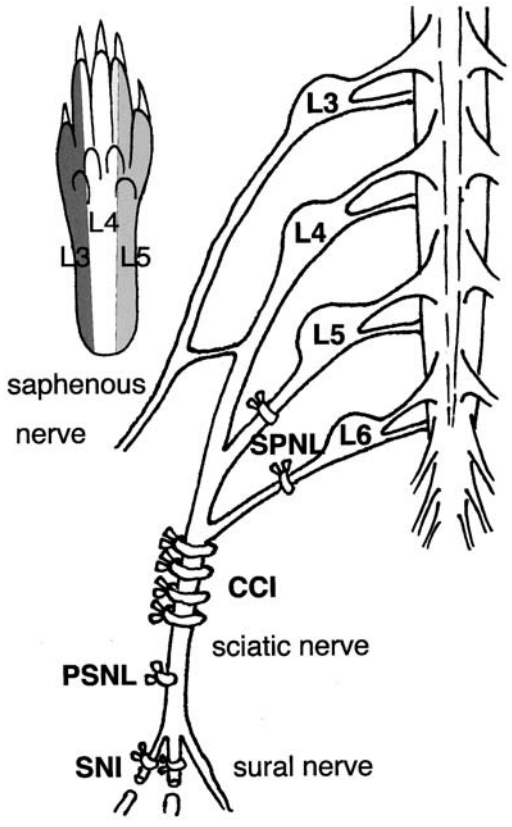

Fig. 1. Schema of the form rat neuropathic pain models. Four loose ligatures using chromic catgut are tied around the sciatic nerve for the chronic constriction injury (CCI) model. Silk suture is used for other three models. The spinal nerve ligation (SPNL) model involves tight ligation of the L5 (and L6) spinal nerve(s). The partial sciatic nerve ligation (PSNL) model involves tight ligation of one-half to one-third of the sciatic nerve. The spared nerve injury (SNI) involves tight ligation and cut of the two main branches of the sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact. The plantar surface of the rat hindpaw is innervated by components of the L3, L4, and L5 spinal nerves.

Table 1
Comparison of the Phenotypic Change in DRG Neurons Following Various Injuries

Molecules	Peripheral Inflammation	Neuropathic pain		
		Directly injured	Spared	ND
Substance P (PPT)	↑ *(10)*	↓ (↑ L) *(71)*	↑ S,M in SPNL ↑ in CCI & PSNL *(11,12)*	
CGRP	↑ *(10)*	↓ (↑ L) *(74)*	↑ S,M in SPNL *(13)*	
BDNF	↑ *(2,24)*	↓ or →S, ↑ M,L *(27,28)*	↑ S,M in SPNL *(29,30)*	↑ in CCI *(30)*
VR1	→ *(40)*	↓ *(39)*	↑ S,M in SPNL ↑ A,C in SPNL ↑ in PSNL *(12,41,42)*	
$P2X_3$	↑ *(52)*	↓ *(50)*	↑ in SNI → in SPNL *(42,54)*	↑ in CCI *(53)*

↑, upregulation; ↓, downregulation; →, no change; A, neurofilament positive neurons; C, neurofilament negative neurons; S, small-sized neurons; M, medium-sized neurons; L, large-sized neurons; CCI, chronic constriction injury of the sciatic nerve; SPNL, spinal nerve ligation; SNI, spared nerve injury; PSNL, partial sciatic nerve ligation; (number), reference number; ND, sparing of neurons was not determined.

nerves *(6)*, most electrophysiological and neurochemical studies have been performed on L4 and L5 DRG neurons. Among these neuropathic pain models, the SPNL model is unique because the uninjured/spared L4 DRG neurons are clearly separated from the injured/axotomized L5 DRG neurons, whereas the injured and uninjured neurons are intermingled in the L4 and L5 DRG in the other three models.

The primary afferent neurons that suffered injury to their peripheral axons increase or decrease their expression of a variety of molecules including receptors, ion channels, and neuropeptides. Indeed, some of these changes may contribute to the pathomechanisms of neuropathic pain, such as spontaneous discharge of these neurons *(8,9)*. However, it is certain that natural stimuli are transferred through the neurons spared from axotomy, because the axotomized neurons can no longer respond to the stimuli applied to peripheral tissues. In the past five years, data on the phenotypic change of the spared primary afferent neurons in the aforementioned neuropathic pain models have been reported. Interestingly, these data are similar to those obtained from peripheral inflammation models, and are suitable in explaining the pathomechanisms of neuropathic pain at the primary afferent level (*see* Table 1).

Substance P and CGRP (and Other Neuropeptides)

Substance P and CGRP are synthesized in 10–30% and 45–70% of DRG neurons, respectively. Peripheral inflammation increases the expression of substance P and CGRP in DRG neurons *(10)*. Preprotachykinin mRNA encoding the protein precursor of substance P increases in the spared DRG neurons following

PSNL and L5 SPNL *(11,12).* CGRP mRNA also increases, whereas mRNAs for galanin, vasoactive intestinal peptide and neuropeptide Y do not increase, in the spared L4 DRG neurons following L5 SPNL *(13).*

Substance P and CGRP are transferred to both peripheral and central terminals of their axons. In the periphery, they are released by depolarization of the axonal terminals, and induce degranulation of mast cells, vasodilation, and extravasation of plasma contents *(14).* These responses, known as neurogenic inflammation, can cause peripheral sensitization of the primary afferents. In the dorsal horn of the spinal cord, these neuropeptides are released with glutamate, and activate and/or sensitize the dorsal horn neurons *(15,16).* Substance P acts on NK1 receptor, and the activation of this G protein-coupled receptor can induce the phosphorylation of the NMDA receptor, which is known as one of the mechanisms of central sensitization of the spinal dorsal horn neurons *(17).* As mentioned earlier, CGRP has functions similar to substance P. However, the contribution to central sensitization of CGRP has still not been directly demonstrated. CGRP acts on at least two subtypes of G protein-coupled receptors, the CGRP1 receptor and CGRP2 receptor *(18).* The former and its associated proteins have been identified *(19–21),* but the latter has not. Although the expression of CGRP receptor(s) in DRG neurons has not been reported, given that CGRP enhances the release of transmitters from DRG neurons by increasing Ca^{2+} conductance *(22),* CGRP receptor(s) can be also expressed in DRG neurons. In addition, the NK1 receptor exists in a subpopulation of DRG neurons *(23).* Thus, substance P and CGRP may urge synaptic transmission in an autocrine or paracrine manner.

BDNF

BDNF, a member of the neurotrophin family, is synthesized in 13–45% of naive DRG neurons *(24–26).* Peripheral inflammation upregulates the expression of BDNF in a nerve growth factor (NGF) dependent manner *(23,24).* On the other hand, peripheral axotomy decreases or does not influence the intensity of BDNF-immunoreactivity in small-sized DRG neurons, and induces *de novo* expression of BDNF in medium- and large-sized neurons *(27,28).* BDNF increases in the small- and medium-sized neurons in the spared L4 DRG following L5 SPNL model *(29,30).* The increase in BDNF expression is very consistent with the development and maintenance of heat hyperalgesia *(29).* Ha and colleagues have also reported the increase in BDNF-immunoreactive DRG neurons following CCI, although they did not identify whether the neurons were injured or spared *(30).*

BDNF synthesized in DRG neurons is transported to the central terminals of the primary afferents in the spinal dorsal horn *(26,31),* is released and acts on the TrkB receptor, a high-affinity BDNF receptor, on second-order sensory neurons. The activation of this receptor tyrosine kinase is known to cause phosphorylation of the NMDA receptor in the hippocampus *(32),* and this phosphorylation is supposed to be one of the mechanisms of central sensitization of the spinal dorsal horn neurons *(17).* Blocking endogenous BDNF by intrathecal infusion of anti-BDNF antibody prevents the development of heat hyperalgesia after L5 SPNL *(29).* On the other hand, BDNF may also act in the peripheral tissues and/or in a paracrine manner, because the TrkB receptor is expressed in a subpopulation of DRG neurons themselves *(33).* In fact, BDNF injection into the rat hindpaw induces thermal hyperalgesia *(34),* and exogenous BDNF directly delivered to the intact DRG causes mechanical allodynia *(35).*

VR1 (Vanilloid Receptor Subtype 1)

VR1 is expressed by 25–50% of naive DRG neurons and the expression of this channel in the DRG is restricted to unmyelinated small- and medium-sized neurons *(36–39).* Peripheral axotomy decreases the expression *(39),* whereas peripheral inflammation seems not to influence the expression *(40).* VR1 increases in the spared

DRG neurons in the L5 SPNL and PSL models *(12,41).* The time course of this upregulation is well-correlated with the development and maintenance of thermal hyperalgesia *(42).*

This nonselective cation channel is the first identified capsaicin receptor *(43).* After synthesized in the neuronal cell body in the DRG, this receptor is transported to both the central and peripheral terminals of the axons. At the peripheral primary afferent terminals, this receptor is thought to be one of the molecular substrates for heat transduction *(44).* The functional significance of the VR1 transportation to the central terminals in the spinal dorsal horn is still unclear, whereas several endogenous chemicals, such as anandamide, 12-15-(S)-hydroperoxyeicosatetraenoic acids, 5-15-(S)-hydroxyeicosatetraenoic acids, and leukotriene B4, directly activate the VR1 *(45–47).* In any case, the increase in VR1 expression in the spared DRG neurons in neuropathic pain models should cause the accumulation of this receptor at the axonal terminals and can cause an exaggerated sensory transmission through these neurons.

$P2X_3$

$P2X_3$ is a member of ionotropic ATP receptors, P2X receptors. An electrophysiological study suggests that the $P2X_3$ homomeric receptor in small-sized DRG neurons and the $P2X_2/P2X_3$ heteromeric receptor in medium-sized neurons are involved in the activation of primary afferents by ATP *(48).* Because ATP is contained in all cells throughout the body, tissue damage should increase local extracellular ATP concentration and activate primary afferent terminals through P2X receptors. Also, because ATP is a co-transmitter of sympathetic postganglionic fibers, ATP and P2X receptors may contribute to sympathetic-sensory coupling. $P2X_3$ is expressed in about 30–55% of all DRG neurons *(49–51).* Peripheral axotomy downregulates *(50)* and peripheral inflammation upregulates the expression of $P2X_3$ in the DRG *(52).* CCI increases $P2X_3$ immunoreactivity *(53),* and SNI increases $P2X_3$ mRNA in the spared DRG neurons *(54).* However, the spared L4 DRG neurons do not change the expression of $P2X_3$ following L5 SPNL *(42).* There seems to be differences in the phenotypic changes of the spared DRG neurons and pathomechanisms between the various neuropathic pain models. Although P2X receptors exist not only on the central terminals of primary afferents but also in the spinal neurons (for review, *see* ref. *55*), recent two studies suggest that these central purinoceptors have limited contribution to pathomechanisms of neuropathic pain *(56,57).* On the other hand, Park et al. demonstrated that systemic injection of phentolamine, an alpha-adrenoceptor blocker, and suramin, a nonselective purinoceptor blocker, attenuated mechanical allodynia at least in a subpopulation of neuropathic rats induced by L5 and L6 SPNL, suggesting peripheral contribution of purinoceptors *(58).* However, the difference of the contribution of purinoceptors among various neuropathic pain models has not been directly demonstrated yet. In any case; the lack of subtype-specific ligands for purinoceptors so far limits such studies.

A Possible Mechanism of the Phenotypic Change in the Spared Neurons

In the L5 (and L6) SPNL model, the spared L4 primary afferents commingle with the degenerating L5 afferents in the sciatic nerve. Therefore, some molecules associated with Wallerian degeneration may have an influence on the adjacent spared nerves *(59).* On the other hand, small-sized DRG neurons are roughly divided into two major classes according to their sensitivity to NGF and glial cell line-derived neurotrophic factor (GDNF) *(60).* Because NGF-sensitive neurons have two traditional neuropeptides; substance P and CGRP, these neurons are called "peptidergic" neurons. These neurons also express the high-affinity NGF receptor, TrkA. Most of the

BDNF-expressing neurons are also members of this class *(61).* On the other hand, GDNF-sensitive neurons that express GFRalpha1 and Ret, the ligand-binding domain and the tyrosine kinase domain of GDNF receptor complex, respectively, are called "non-peptidergic". $P2X_3$-expressing neurons are contained mainly in this class *(50,51).* VR1 is expressed in both the peptidergic- and non-peptidergic classes *(39,44).* Consistent with this classification, NGF increases substance P, CGRP, and BDNF-expression *(10,61),* and GDNF increases $P2X_3$-expression in the DRG *(50).* VR1-expression is regulated by both NGF *(62)* and GDNF *(63).* Therefore, all four molecules demonstrated to increase in the spared L4 DRG neurons following L5 SPNL could be regulated by NGF. In fact, NGF content increases transiently in the sciatic nerve and later in the L4 DRG following this type of injury *(29).* However, our experimental data does not support this hypothesis. The increase in BDNF and VR1 mRNAs in the spared L4 DRG and the development of behavioral hypersensitivity precede the increase in NGF in the DRG *(29,42),* whereas those in PPT and CGRP mRNAs coincide with the increase in NGF *(12).* Sequestering of NGF by local application of an antibody prevented the development of thermal hyperalgesia, but not the increase in BDNF in the L4 DRG *(29).* Thus, some other factors may contribute to the phenotypic changes in the spared neurons.

Behavioral and Electrophysiological Evidences

Previous studies demonstrated that dorsal rhizotomy of the directly injured L5 DRG prevented or reversed mechanical allodynia after L5/6 SPNL *(64,65).* Indeed, multiple pathomechanisms should contribute to the development and maintenance of neuropathic pain. Abnormal anatomical and physiological changes can occur in the directly injured DRG neurons and their fibers, such as spontaneous firing and sensory-sympathetic coupling *(9, 66).* However, recent studies failed to confirm the effect of dorsal rhizotomy of the directly injured neurons, while they used a more simplified L5 SPNL *(59,67).* As mentioned earlier, given that the directly injured L5 primary afferents do not have anatomical connections to the peripheral tissues, the spared L4 afferents should be the only route to conduct the peripheral information from the plantar surface of the hindpaw to the spinal cord in this neuropathic pain model.

It has been suggested that central sensitization of the dorsal horn neurons has an important role for induction of abnormal pain-related behaviors. Spontaneous discharges derived from the directly injured primary afferents and their cell bodies may activate central mechanisms that have been sensitized by the injury and may cause spontaneous pain. However, this does not explain stimulus-evoked allodynia and hyperalgesia. Central sensitization requires a prolonged activity of C-fibers containing excitatory neuromodulators, such as substance P and BDNF *(17).* However, the only spontaneous discharges recorded from the directly injured L5 nerve are derived from the A-fibers in the L5 SPNL model *(9).* Spontaneously firing DRG neurons have been also found in the spared L4 DRG following L5 SPNL *(8),* and more importantly, spontaneously active C-fibers have been recorded from the L4 spinal nerve in this model *(68)* (also see *69,70*). Because these C-fibers terminate in the superficial dorsal horn, and substance P and BDNF increase in the small-sized spared DRG neurons, these primary afferent neurons have the potential to induce central sensitization in pain pathways (Fig. 2B).

Finally, we mention a possible contribution of the spontaneously active A-fibers in the directly injured L5 nerves in this model. Substance P and BDNF have been demonstrated to increase in a subpopulation of the directly axotomized large-sized DRG neurons *(71,72).* If these neurons are the source of the spontaneous discharge, central sensitization could occur in the non-noxious sensory pathways *(27,28, 71)* (Fig. 2D,E). Furthermore, peripheral axotomy has

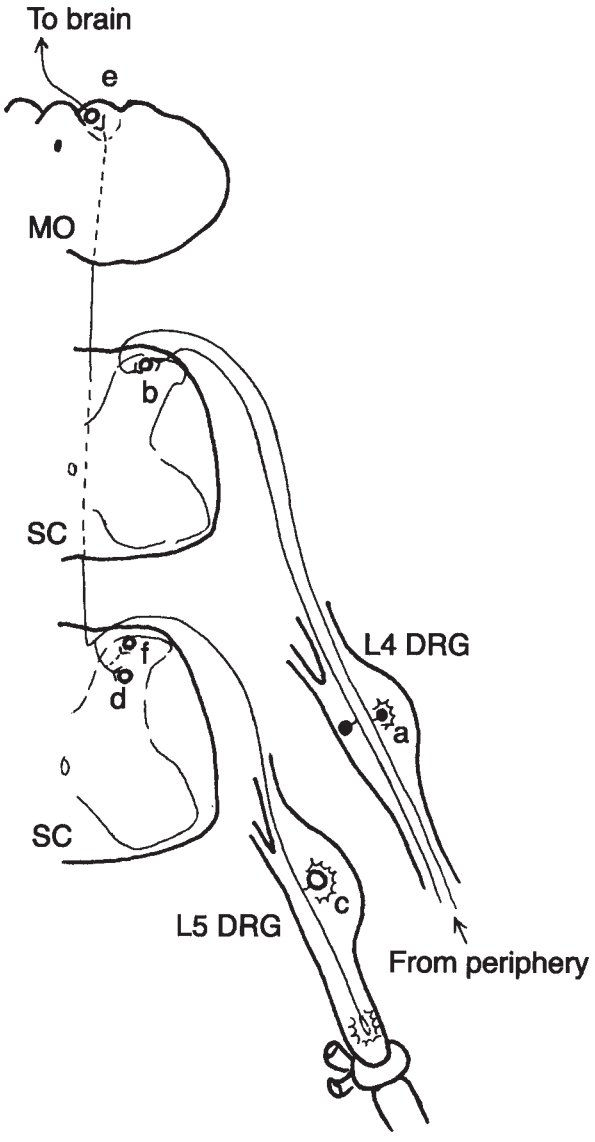

Fig. 2. Spontaneous discharges of primary afferent neurons and their possible contribution to central sensitization following application of the L5 spinal nerve ligation model. In the spared L4 DRG, the spontaneous discharge of C-fibers **(a)** and the increase in substance P and BDNF, two important neuromodulators for central sensitization, have been found. These changes are suitable as mechanisms of sensitization of the secondary neurons in the superficial layer of the dorsal hom **(b)** in spinal cord (SC) mediating noxious stimuli conduction to upper centers. In the directly injured L5 DRG, spontaneous discharge has been found only in A-fibers **(c)** terminating in the deeper layer of the dorsal horn **(d)** and in the dorsal column nucleus **(e)** in the medulla oblongata (MO). Because a subpopulation of the directly injured A-fiber neurons increase the expression of substance P and BDNF, this spontaneous firing may influence the secondary neurons in these areas normally mediating innocuous stimuli. In addition, central sprouting of the axotomized A-fibers has been demonstrated. This structural reorganization could cause new connections between spontaneous A-fibers and the pain-mediating superficial dorsal horn neurons **(f).**

been proposed to induce central sprouting of A-fibers into the superficial layer of the dorsal horn *(73).* This structural reorganization could cause sensitization of the neurons in the superficial dorsal horn by axotomized spontaneously firing A-fibers (Fig. 2F).

In conclusion, peripheral nerve injury induces phenotypic and physiological changes, not only in the directly injured primary afferent neurons, but also in the spared ones, whereas the changes are qualitatively different between these neurons. At present, unknown interactions between directly injured neurons and spared neurons seem to occur in the DRG and/or in the peripheral nerve. The mechanism and/or the mediator of these interactions will be a significant issue in future pain research.

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