Scandinavian Journal of Pain 6 (2015) 3–6

Contents lists available at ScienceDirect

Scandinavian Journal of Pain

journal homepage: www.ScandinavianJournalPain.com

Topical review

Perspectives in Pain Research 2014: Neuroinflammation and glial cell activation: The cause of transition from acute to chronic pain?

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HIGHLIGHTS

- The immune system and the pain system are closely linked.
- Glial cells are activated in the dorsal root ganglion and at the spinal cord.
- Glia cells may cause contralateral spreading and possible widespread sensitisation.
- Opioids may stimulate microglia cells to produce proinflammatory cytokines.

ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form 21 October 2014
Accepted 22 October 2014
Available online 24 November 2014

Keywords:
Neuroinflammation
Chronic pain
Widespread sensitisation
Glia
Cytokines

ABSTRACT

Background: It is unknown why an acute pain condition under various circumstances can transition into a chronic pain condition.

There has been a shift towards neuroinflammation and hence glial cell activations specifically in the dorsal root ganglion and spinal cord as a mechanism possibly driving the transition to chronic pain. This has led to a focus on non-neuronal cells in the peripheral and central nervous system. Besides infiltrating macrophages, Schwann cells and satellite glial cells release cytokines and therefore important mechanisms in the maintenance of pain. Activated Schwann cells, satellite glial cells, microglia, and astrocytes may contribute to pain sensitivity by releasing cytokines leading to altered neuronal function in the direction of sensitisation.

Aims of this perspective paper: 1) Highlight the complex but important recent achievement in the area of neuroinflammation and pain at spinal cord level and in the dorsal root ganglion.

2) Encourage further research which hopefully may provide better understanding of new key elements driving the transition from acute to chronic pain.

Recent results in the area of neuroinflammation and pain: Following a sciatic nerve injury, local macrophages, and Schwann cells trigger an immune response immediately followed by recruitment of blood-derived immune cells. Schwann cells, active resident, and infiltrating macrophages release proinflammatory cytokines. Proinflammatory cytokines contribute to axonal damage and also stimulate spontaneous nociceptor activity. This results in activation of satellite glial cells leading to an immune response in the dorsal root ganglia driven by macrophages, lymphocytes and satellite cells. The anterograde signalling progresses centrally to activate spinal microglia with possible upregulation of glial-derived proinflammatory/pronociceptive mediators.

An important aspect is extrasegmental spreading sensitisation where bilateral elevations in TNF-α, IL-6, and IL-10 are found in dorsal root ganglion in neuropathic models. Similarly in inflammatory pain models, bilateral up regulation occurs for TNF-α, IL-1β, and p38 MAPK. Bilateral alterations in cytokine levels in the DRG and spinal cord may underlie the spread of pain to the uninjured side.

An important aspect is how the opioids may interact with immune cells as opioid receptors are expressed by peripheral immune cells and thus can induce immune signaling changes. Furthermore, opioids may stimulate microglia cells to produce proinflammatory cytokines such as IL-1.

DOI of refers to article: http://dx.doi.org/10.1016/j.sjpain.2014.10.004.
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http://dx.doi.org/10.1016/j.sjpain.2014.10.002

Conclusions: The present perspective paper indicates that neuroinflammation and the associated release of pro-inflammatory cytokines in dorsal root ganglion and at the spinal cord contribute to the transition from acute to chronic pain. Neuroinflammatory changes have not only been identified in the spinal cord and brainstem, but more recently, in the sensory ganglia and in the nerves as well. The glial cell activation may be responsible for contralateral spreading and possible widespread sensitisation.
Implications: Communication between glia and neurons is proposed to be a critical component of neuroinflammatory changes that may lead to chronic pain. Sensory ganglia neurons are surrounded by satellite glial cells but how communication between the cells contributes to altered pain sensitivity is still unknown. Better understanding may lead to new possibilities for (1) preventing development of chronic pain and (2) better pain management.

Contents

1. Introduction

Why does acute pain sometimes transition into a disabling chronic pain condition? This simple question continues to confound pain researchers. There has, however, been a gradual shift towards neuroinflammation and hence glial cell activations as key mechanisms that may favor the transition to chronic pain. This has led to a focus on the role of non-neuronal cells in the peripheral and central nervous system. Besides infiltrating macrophages, Schwann cells in the peripheral nerve, satellite glial cells, which are part of the peripheral nervous support system and are found in the dorsal root and trigeminal ganglia, and microglia, the endogenous immune cells of the central nervous system, can release cytokines thought to be an important mechanism in the maintenance of pain in conditions such as neuropathies and inflammatory pain. It is thought that activated Schwann cells, satellite glial cells, microglia, and astrocytes contribute to the maintenance of pain sensitivity by releasing a steady stream of cytokines that lead to altered neuronal function in the direction of sensitisation.

2. Neuroinflammation and neuropathic pain

In murine experimental models of neuropathic pain, it has been demonstrated that, following sciatic nerve injury, local macrophages and Schwann cells trigger an immune response immediately followed by recruitment of blood-derived immune cells. Schwann cells, active resident and infiltrating macrophages release proinflammatory cytokines, including the interleukins (IL)-1β, -6, -12 and tumor necrosis factor-α (TNF) as well as chemokines [1,2]. Proinflammatory cytokines contribute to axonal damage and they also stimulate spontaneous nociceptor activity. The load of inflammatory mediators at the site of nerve injury can provoke spontaneous action potential discharge in nociceptive fibers, causing an amplification of abnormal signals that reach dorsal root ganglion (DRG) sensory neurons and satellite glial cells. This results in activation of satellite glial cells, which leads to an immune response in the dorsal root ganglia that is driven by macrophages, lymphocytes and satellite cells. The anterograde signalling progresses centrally to activate spinal microglia with possible upregulation of glial-derived proinflammatory/pronociceptive mediators [3]. In particular, the increase in IL-1 in the DRG and spinal cord correlates well with the presence of both hyperalgesia and allodynia that is observed in these models [4,5]. However it is also well known that in the peripheral immune system antinflammatory cytokines, such as IL-10, act as endogenous feed-back inhibitors in order to maintain a balanced immune response. A significant alteration of IL-10 expression has been reported both in the peripheral and the central nervous system associated to neuropathic pain development [6]. Strategies aimed at enhancing IL-10 production have consistently resulted in prevention and reversal of pain hypersensitivity in models of neuropathy [7].

3. Bilateral pain spread

One important aspect associated with the development of chronic pain in humans is the contralateral spreading of sensitisation and later the widespread sensitisation. Most animal studies on localized experimental trauma have not systematically assessed ipsilateral and contralateral extraterritorial reactions and sensitisation in areas supplied by unaffected heteronymous nerves. For those that have studied this phenomenon, the predominant findings have been that peripheral inflammation or nerve lesions affect the non-inflamed or non-lesioned structures [8]. Although the underlying mechanisms are not fully understood, various models for the spreading have been implemented to study mechanisms such as up-regulation of neuro-inflammatory reaction (cytokine responses) and glial cell activation [9–12].

Bilateral elevation of TNF-α and IL-10 in dorsal root ganglion (DRG) associated with a unilateral injured spinal nerve has been found [13,14]. Interestingly, IL-10 may also prevent or reverse many pathological pain states, including pain induced by chronic constriction injury neuropathies [15]. Bilateral elevation of IL-6 protein in the DRG after spinal nerve injury has also been found [16]. Similarly in inflammatory pain models, bilateral changes occur; for example, TNF-α, IL-1β, and p38 MAPK expression are activated after hindpaw but not forepaw carrageenan injection, suggesting segmental mechanisms which so far are not known in detail [17]. These results obtained in inflammatory and neuropathic pain models suggest that bilateral alterations in cytokine levels in the DRG

and spinal cord may underlie the spread of pain to the uninjured side.

After a nerve ligation rats develop mechanical and cold allodynia in the contralateral paw [18] and show elevated bilateral IL-6 and activator of transcription 3 (STAT3) signaling in satellite glial cells [19]. IL-6 contributes to the survival of axotomized neurons, growth and differentiation of neurons, formation of dendrites, neurotransmitter metabolism, and other processes and is able to promote axonal regeneration [20], but there is evidence that it also contributes to the development of neuropathic pain [21]. After chronic constriction injury of the sciatic nerve in rats, tactile allodynia in the hindpaw territories of both the injured sciatic nerve and the uninjured saphenous nerve is shown [22]. These data support the growing body of evidence that not only neurons supplying the inflamed or injured site but also uninjured primary sensory neurons play a role in inflammatory and neuropathic pain induction [23,24].

Spinal glial processes appear to be important contributors to the bilateral spread of inflammatory pain as well. For example, hindpaw injection with 4% carrageenan causes both ipsilateral and contralateral allodynia with the contralateral allodynia correlating to the increase in spinal OX-42 immunoreactivity; a measure of microglial activation. Intrathecal treatment with a microglial inhibitor (minocycline) inhibits the developed contralateral allodynia [25]. Similar contralateral microglial proliferation occurs after nerve injury [26–28] and injection of 5% formalin into the hindpaw [29]. Also a paw burn injury causes phospho-p38-expressing microglial activation bilaterally in the spinal cord dorsal horn and produces long-lasting bilateral allodynia [30] suggesting that a variety of local pain provocation tests can cause bilateral microglial activation and sensitisation.

4. Ganglion neuron-satellite glial cell communication

Communication between glia and neurons has been proposed to be a critical component of neuroinflammatory change and alteration in pain sensitivity that leads to chronic pain. In sensory ganglia, neurons are completely surrounded by satellite glial cells, however, how communication between these two cells contributes to altered pain sensitivity is just beginning to be unraveled.

Laursen et al. investigated whether satellite glial cells release glutamate and whether elevation of trigeminal ganglion glutamate concentration alters response properties of trigeminal afferent fibers that innervate muscle [31]. These satellite glial cells were found to express excitatory amino acid transporters and could be stimulated to release glutamate. Intraganglionic injections of glutamate evoked neuronal discharge and significantly reduced the mechanical activation threshold of the peripheral endings of the nerve fibers. This effect of glutamate on trigeminal ganglion neurons was mediated through activation of N-methyl-D-aspartate (NMDA) receptors. These findings provide a novel mechanism whereby activation of satellite glial cells could contribute to cranial muscle tenderness in craniofacial pain conditions such as migraine headache.

5. Toll-like receptors

De Goeij et al. investigated pro-inflammatory cytokine recruitment by giving heathly humans *Escherichia coli* endotexemia, which causes flu-like symptoms. Treatment increased plasma levels of cytokines, including tumour necrosis α and interleukins, reduced electrical and pressure pain thresholds by 10–20% and decreased tolerance to the cold pressor test [32]. There was, however, no relationship between cytokine levels and changes in pain sensitivity. Endotoxins promote cytokine release through an interaction with Toll like receptors (TLR) found on peripheral immune cells. Glia can also be activated by these receptors and it has been proposed that this contributes to chronic pain through the release of pro-inflammatory cytokines in the central nervous system. Kwok et al. examined TLR responsiveness in peripheral immune cells in vitro [33]. Agonist activation of TLR2 increased interleukin (IL)-1β release in chronic pain patients, but had no effect on pain free patients. Agonist activation of TLR4 increased IL-1β release in all subjects, but increased responsiveness was seen in the chronic pain patients. Release of IL-1β was greater in patients receiving opioid analgesics than in those who were not receiving these drugs.

5.1. The effect of opioids on the immune cells

The relationship between opioid receptors, opioid agonists and TLRs is an intriguing story. Opioids, such as morphine, interact with opioid receptors expressed by peripheral immune cells and thus can induce immune signaling changes [6]. Morphine induces microglial activation through its action on the TLR4 expressed in microglia, leading to the release of cytokines, including IL-1β, and suppression of the analgesic effect of morphine [6,34].

Furthermore Sacerdote et al. reported that in mice, morphine reduced the expression of TLR4, but not TLR2, by peritoneal macrophages both in vitro and in vivo, and this decrease was mediated through activation of μ opioid receptors expressed by macrophages [6]. In contrast, morphine appears to potentially directly interact with TLR4, independently on μ opioid receptor, to stimulate microglia cells to produce proinflammatory cytokines such as IL-1, that may have a role in the long term in sustaining central neuroinflammation and in diminishing the analgesic effect of opioids [6].

Development of opioid tolerance is a clinical issue which can be difficult to deal with and a better understanding is needed to prevent it from developing. It has recently been suggested that the activation of glial cells (including astrocytes and microglia) in the spinal cord plays an important role in the development of opioid tolerance [35,36].

The effect of the microglial inhibitor minocycline attenuated the development of morphine tolerance in mice [37]. Interestingly, taken together, these results indicate a differential effect of morphine on cytokine production in the periphery and CNS.

5.2. G protein-coupled receptor kinase 2

One potentially important regulator of the transition from acute to chronic pain that has come to light recently is the G protein-coupled receptor kinase 2 (GRK2). This enzyme acts to desensitize agonist occupied G protein coupled receptors (GPCRs) by uncoupling the receptor from its G proteins and by facilitating the internalization of the receptor to protect cells from overstimulation [38]. GRK2 is found in the nervous system and immune system, and deficiency of this receptor markedly prolongs inflammatory pain without an effect on basal sensory thresholds. In neuropathic and inflammatory injury, the expression of GRK2, particularly in immune cells (microglia and macrophages in the spinal cord) is substantially lowered, which is proposed to lead to an increased agonist response of GPCRs [38]. Transgenic mice with low GRK2 expression (~50% less GRK2 than wild type) develop long-lasting hyeralgesia that out lasts the inflammation caused by carrageenan and other inflammatory mediators injection into the paw [38].

Wang et al. looked at whether reduction of GRK2 in dorsal root ganglion also contributes to the transition from acute to chronic pain [39]. The study used models of "hyperalgesic" priming where an inflammatory stimulus like carrageenan is used to induce inflammation, followed 7 days later by a single insult of an algogenic compound, such as prostaglandin E2 (PGE2), which results in a

prolonged mechanical sensitisation. Hyperalgesic priming was found to significantly decrease the level of GRK2 in DRG neurons thought to mediate nociceptive signaling; an effect that lasted until resolution of the primed state. Infection of the mice with a herpes simplex virus vector that encoded bovine GRK2 substantially shortened the duration of hyperalgesia after PGE2, suggesting that elevating GRK2 inhibits the priming effect. These findings suggest that maintaining high levels of GRK2 in the dorsal root ganglia may prevent the development of chronic pain after acute injury and could even be used to treat established chronic pain. Repeated surgeries, where priming by repeat tissue insult may be associated with a transition from acute to chronic pain, may be one example of where this treatment may ultimately prove valuable.

6. Conclusions

These recent results indicate that neuroinflammation and the associated release of pro-inflammatory cytokines contributes to the transition from acute to chronic pain. Neuroinflammatory changes have not only been identified in the sensory neurons of the dorsal root ganglia, spinal cord, and brainstem, but more recently, in the sensory ganglia and in the nerves as well. This latter finding is intriguing, since the peripheral nervous system is more accessible than the central nervous system for many potential therapeutic agents. The glial cell activation in the dorsal root ganglion, and spinal cord may be responsible for contralateral spreading and possible widespread of sensitisation. Peripheral non-neuronal cells need to be considered either to better understand and possibly overcome the limitations of classical analgesics, such as in opioid therapy, or as a novel target for the development of drugs to ameliorate chronic pain.

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