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Targeting microglial purinergic signaling to improve morphine analgesia

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Accumulating evidence indicates an important role of spinal cord microglial cells in the genesis of nerve injury-induced neuropathic pain [7;13;16]. In particular, the ATP receptor P2X4 is up-regulated in spinal cord microglial cells after peripheral nerve injury, and intrathecal injections of P2X4 antisense oligodeoxynucleotides (asODN) attenuate nerve injury-induced tactile allodynia [18]. Consistently, nerve injury-induced tactile allodynia is also markedly attenuated in P2X4 knockout mice [17]. Further studies have revealed a signaling pathway by which P2X4 up-regulation in microglia can facilitate neuropathic pain via interaction with neurons (Fig. 1). Activation of P2X4 in microglia by ATP results in phosphorylation of p38 mitogen-activated protein kinase (MAPK) [15], which has been shown to be critical for microglial signaling and neuropathic pain sensitization [6]. Activation of p38 by phosphorylation leads to the synthesis and release of several “glial mediators”, such as the proinflammatory cytokines IL-1 β and TNF- α [6;9], and the neurotrophin BDNF [15]. These mediators have been shown to modulate both excitatory and inhibitory synaptic transmission in the spinal cord nociceptive circuitry, leading to an increase in pain sensitivity [3;8] (Fig. 1). Of note, microglial responses in the spinal cord are also implicated morphine tolerance after chronic exposure of morphine [12;19]. Is P2X4-triggered microglial signaling also involved in morphine tolerance?

In this issue of *Pain*, Horvath et al. [5] present several lines of compelling evidence that demonstrates a critical role of P2X4 in morphine tolerance in rats. First, persistent morphine infusion via osmotic minipumps induce a marked increase in spinal P2X4 levels, and P2X4 is co-expressed with microglial surface marker CD11b. Second, morphine infusion induces an up-regulation of μ opioid receptor (μ OR) in the dorsal horn, and Confocal analysis shows that some μ OR is expressed in microglial processes. Third, intrathecal injections of P2X4 asODN inhibit morphine-induced P2X4 receptor expression. Importantly, the asODN treatment almost completely prevents the development of antinociceptive tolerance to systemically administered morphine. Thus, P2X4 signaling in spinal cord microglia modulates spinal cord neuronal plasticity underlying both neuropathic pain sensitization and morphine tolerance. Although not directly tested in this study, activation of P2X4 receptor may elicit morphine tolerance by producing glial mediators TNF- α , IL-1 β , and BDNF via p38 activation (Fig 1), since intrathecal infusion of p38 inhibitor has also been shown to prevent morphine tolerance [1;4]. To further confirm the specific role of P2X4 in morphine tolerance, P2X4 knockout mice should be tested for morphine tolerance. P2X4-GFP mice will also be very helpful to confirm P2X4 expression in microglia.

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Competing interest

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Many groups in the pain field used changes in microglial surface markers such as CD11b (labeled by the antibody OX-42) and Iba1 to represent “microglial activation”. Although CD11b and Iba1 are very specific markers for microglia and highly regulated under various injury conditions and after prolonged morphine exposure, use of these surface markers as sole indication for microglial activation could be misleading. Previous work from DeLeo’s group has demonstrated dissociation between CD11b expression and neuropathic pain behavior after nerve injury [2]. So far, we do not have direct evidence showing that these surface markers can directly regulate pain sensitivity. More importantly, we do not know how CD11b and Iba1 regulate the production and release of glial mediators, although these surface markers may regulate microglial reaction or microgliosis. Indeed, Horvath et al. are very careful to avoid the use of the term “glial activation”. Instead, they used the term “glial reactivity”, defined as increased glial hypertrophy, migration and release of pro-inflammatory factors [5]. Of interest the paper by Horvath et al. has also shown that P2X4 asODN treatment can abolish morphine-induced Iba1 increase in the spinal cord [5]. In contrast, P2X4 asODN treatment did not affect nerve injury-induced CD11b expression in the spinal cord [18]. This discrepancy may result from different animal models (nerve injury versus morphine tolerance) and different surface markers (CD11b versus Iba1). It remains to be investigated whether and how Iba1 expression contributes to morphine tolerance.

Another interesting finding in the paper by Horvath et al. is P2X4 asODN treatment increases the perivascular microglial marker ED2 whereas sustained morphine exposure reduces ED2 expression in the spinal cord [5]. In parallel, previous study from this group has shown that peripheral nerve injury reduces ED2 expression and cannabinoid receptor 2 agonist increases ED2 expression. Notably, up-regulation of ED2 by cannabinoid receptor 2 agonist is associated with a reduction of nerve injury-induced tactile allodynia [14]. It is well known that microglial cells play both detrimental and protective roles in neurodegenerative conditions [11]. The protective role of glial cells in chronic pain has also been discussed in a recent review article [10]. Thus it is of great importance to examine whether there are different types microglia that play respective pro-inflammatory and anti-inflammatory roles in chronic pain conditions. It is also important that we can find new ways to convert “bad” microglia (pro-inflammatory) to “good” microglia (anti-inflammatory and pro-resolving). As discussed in the paper by Horvath [5], further studies are needed to determine the role of ED2 and perivascular microglia in the resolution of neuropathic pain and recovery from morphine tolerance. However, caution must be taken, since ED2-expressing macrophages/microglia are regarded as resident macrophages/microglia and these cells express pro-inflammatory mediators such as COX-2 and IL-1 β . Whether these cells possess anti-inflammatory features only in the recovery phase of injuries needs further investigation.

Targeting microglial signaling with P2X4 antagonists holds promise for the treatment of chronic pain. Given the fact that microglia play both detrimental and protective role in chronic pain conditions, it is conceivable to target a specific signaling pathway in microglia without impairing other cellular functions of microglia. These microglia-targeting P2X4 antagonists may be developed to attenuate chronic pain directly or indirectly by improving the analgesic efficacy of opioids. The new information presented in the work by Horvath’s et al. will advance our understanding of this target and help future clinical development.

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Figure 1. Schematic showing P2X4-mediated intracellular signaling in spinal cord microglia in conditions of nerve injury and chronic opioid exposure.