This Week in The Journal

Low-Dose Morphine Sensitizes Pain through TLR4

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(see pages 6414–6424)

Opioids powerfully quell pain but also paradoxically increase pain sensitivity in animals and people. This week, Araldi et al. examined the molecular mechanisms responsible for the phenomena termed opioid-induced hyperalgesia and hyperalgesic priming in rats. Naïve rodents injected with the proinflammatory mediator prostaglandin E₂ (PGE₂) develop pain sensitivity that resolves within an hour. In contrast, when animals receive and then recover from a painful “priming” insult days beforehand, they continue to show signs of pain for hours or days after a PGE₂ injection. Opioids can serve as such a priming insult, working at least in part through receptors other than the μ -opioid receptor (MOR) that mediates opioids’ analgesic effects.

Araldi et al. hypothesized that “priming” with opioids might involve the same underlying mechanisms as a priming injury, so they focused on toll-like receptor 4 (TLR4), an immune receptor previously implicated in hyperalgesic priming. They injected male rats intrathecally with antisense oligodeoxynucleotides (AS-ODN) to TLR4 or a control ODN for 3 d; on the fourth day they treated rats with low-dose morphine (0.03 mg/kg). Control rats displayed opioid-induced hyperalgesia, but those with reduced TLR4 did not. The next day, rats received an injection of PGE₂ and their responses to mechanical stimuli were later tested. Hyperalgesic responses were still apparent after 4 h in rats treated with low-dose morphine alone, but not in rats treated with morphine and AS-ODN for TLR4, indicating that TLR4 receptors mediated both hyperalgesia phenomena. Further experiments demonstrated that hyperalgesia also depended on protein kinase C epsilon. High-dose morphine (3 mg/kg) produced strong analgesia in rats with or without AS-ODN

for TLR4. Hyperalgesic priming—prolonged painful sensitivity following PGE₂ injection—was also evident in both groups of rats, indicating the higher dose of morphine caused priming independent of TLR4. Furthermore, when either or both of two major classes of nociceptive sensory neurons were ablated, low-dose morphine no longer produced hyperalgesia on its own, but morphine-induced hyperalgesic priming remained mostly intact. Ablation of the sensory neurons also prevented priming by high-dose morphine. The work provides new insights about how opioids sensitize neurons to detect pain, and how the drugs might best be used to treat chronic pain and opioid use disorder.

Sensory neurons from DRG (green) contain DNMT1 in the nuclei (red).

Epigenetic Regulation of Kv1.2 by DNMT1 in Neuropathic Pain

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(see pages 6595–6607)

Sensory neurons of the dorsal root ganglia (DRG) provide spinal neurons with sensory information, which they collect via long processes that extend to the far reaches of the body. Those nerves are vulnerable to injury from a host of threats, and damage to them leads to neuropathic pain. That process entails silencing genes

encoding ion channels and other signaling proteins in DRG neurons.

This week, Sun, Gu, et al. identify DNA methyl transferase 1 (DNMT1) as a key molecule responsible for generation of neuropathic pain. Previous work showed that methylation activity by DNMT3 silenced genes and contributed to neuropathic pain whereas DNMT1 was considered a maintenance enzyme. Here, the authors first showed that DNMT1 was expressed in the DRG and upregulated by cAMP response element-binding protein (CREB) after spinal nerve ligation (SNL) injury, a model of neuropathic pain in mice. The mechanical, cold and heat pain hypersensitivities seen after nerve injury were reduced in mice that received the DNMT inhibitor RG108.

The authors next knocked down DNMT1 specifically in the fourth lumbar (L4) DRG, and they also generated mice in which DNMT1 was knocked out specifically from sensory neurons, effectively removing DNMT1 from sensory ganglia while leaving DNMT3 expression intact. Mice lacking DNMT1 displayed less pain hypersensitivity following nerve injury than did controls, but sensory and motor responses were intact following sham surgery. A week after injury, several key proteins were downregulated in the DRG including Kv1.2, a voltage-gated potassium channel that regulates neuronal excitability. Knockdown of DNMT1 in L4 DRG reversed that downregulation, pointing to silencing of *Kcna2* by DNMT1. Whole-cell recordings from DRG neurons cultured 7 d after SNL showed significantly reduced Kv current density, increased resting membrane potential, and decreased action potential threshold compared to sham-operated mice; those changes were absent or much smaller in neurons lacking DNMT1.

The study suggests that restoring Kv1.2 expression silenced by DNMT1 might be a viable way to alleviate nerve hyperexcitability in neuropathic pain.

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