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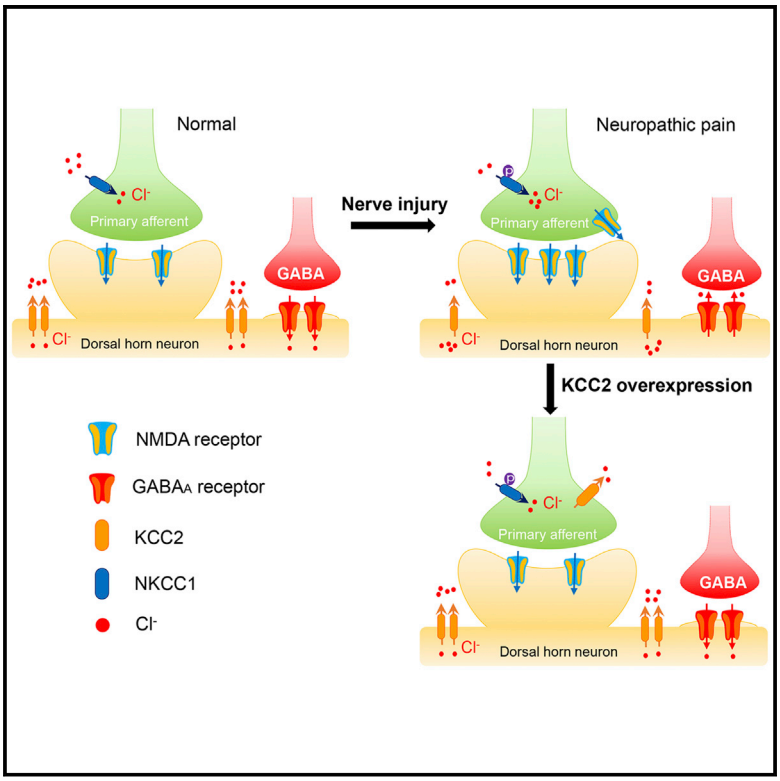
Chloride Homeostasis Critically Regulates Synaptic NMDA Receptor Activity in Neuropathic Pain

Graphical Abstract



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In Brief

Li et al. find that spinal KCC2 gene transfer induces sustained KCC2 expression and restores chloride homeostasis disrupted by nerve injury in both dorsal horn and primary sensory neurons. KCC2 gene transfer completely and persistently eliminates neuropathic pain and normalizes pre- and postsynaptic NMDA receptor activity increased by nerve injury.

Highlights

- Intrathecal delivery of KCC2 in lentiviral vectors eliminates neuropathic pain
- KCC2 gene transfer restores spinal cord KCC2 function impaired by nerve injury
- KCC2 ectopic expression counteracts NKCC1 activity in primary sensory neurons
- Restoring Cl^- homeostasis normalizes spinal cord synaptic NMDA receptor activity

Li et al., 2016, Cell Reports *15*, 1376–1383
May 17, 2016
http://dx.doi.org/10.1016/j.celrep.2016.04.039

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Chloride Homeostasis Critically Regulates Synaptic NMDA Receptor Activity in Neuropathic Pain

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http://dx.doi.org/10.1016/j.celrep.2016.04.039

SUMMARY

Chronic neuropathic pain is a debilitating condition that remains difficult to treat. Diminished synaptic inhibition by GABA and glycine and increased NMDA receptor (NMDAR) activity in the spinal dorsal horn are key mechanisms underlying neuropathic pain. However, the reciprocal relationship between synaptic inhibition and excitation in neuropathic pain is unclear. Here, we show that intrathecal delivery of K^+-Cl^- cotransporter-2 (KCC2) using lentiviral vectors produces a complete and long-lasting reversal of pain hypersensitivity induced by nerve injury. KCC2 gene transfer restores Cl^- homeostasis disrupted by nerve injury in both spinal dorsal horn and primary sensory neurons. Remarkably, restoring Cl^- homeostasis normalizes both presynaptic and postsynaptic NMDAR activity increased by nerve injury in the spinal dorsal horn. Our findings indicate that nerve injury recruits NMDAR-mediated signaling pathways through the disruption of Cl^- homeostasis in spinal dorsal horn and primary sensory neurons. Lentiviral vector-mediated KCC2 expression is a promising gene therapy for the treatment of neuropathic pain.

INTRODUCTION

Chronic neuropathic pain is a major, debilitating clinical problem that remains difficult to treat. Because all of the existing analgesics for treating neuropathic pain, including anti-depressants, opioids, and gabapentinoids, have limited efficacy and often produce intolerable adverse effects (Finnerup et al., 2015; Sommer, 2015), the development of highly effective treatments with minimal off-target effects is urgently needed. GABAergic and glycinergic interneurons, which are densely distributed in the spinal dorsal horn, are the basis of the gate control theory of pain (Melzack and Wall, 1965). Normal synaptic inhibition by GABA and glycine critically depends on the coordinated activities of two functionally distinct cation-chloride cotransporters: Na^+-K^+-$2Cl^-$ cotransporter-1 (NKCC1) and K^+-Cl^- cotransporter-2 (KCC2). KCC2, encoded by *Slc12a5*, is the dominant neuronal Cl^- extrusion mechanism, whereas NKCC1 normally raises intracellular Cl^- levels above equilibrium and opposes the action of KCC2 (Payne et al., 1996; Rivera et al., 1999). Thus, changing the intracellular Cl^- concentration can profoundly alter the strength and polarity of GABA- or glycine-mediated responses. Neuropathic pain caused by peripheral nerve damage, spinal cord injury, diabetic neuropathy, and chemotherapy is associated with reduced KCC2 activity or increased NKCC1 activity in spinal dorsal horn neurons (Boulenguez et al., 2010; Chen et al., 2014d; Coull et al., 2003; Jolivalt et al., 2008; Zhou et al., 2012). Nerve injury also increases NKCC1 phosphorylation and activity in dorsal root ganglion (DRG) neurons to reduce presynaptic inhibition (Chen et al., 2014a; Mòdol et al., 2014). However, it is unclear whether and to what extent restoring Cl^- homeostasis at the spinal cord level leads to long-term reduction in neuropathic pain.

In addition to diminished synaptic inhibition, increased N-methyl D-aspartate receptor (NMDAR) activity in the spinal dorsal horn plays a key role in the development of neuropathic pain (Chaplan et al., 1997; Chen et al., 2014c). Peripheral nerve injury increases spinal NMDAR activity, which impairs synaptic inhibition through calpain-mediated KCC2 proteolysis (Zhou et al., 2012). However, it is unclear whether the loss of Cl^--dependent synaptic inhibition accounts for the increased NMDAR activity in the spinal dorsal horn induced by nerve injury. To determine whether restoring Cl^- homeostasis reduces the synaptic NMDAR activity in the spinal dorsal horn that is increased by nerve injury and, therefore, relieves neuropathic pain, we studied intrathecal KCC2 gene delivery in rat models. Gene therapy offers the potential to correct these sustained abnormal signaling pathways and is well suited for treating chronic pain. Lentiviral vectors in particular have the advantages of long-term transgene expression, low immunogenicity, and the ability to accommodate larger transgenes and transduce nondividing cells such as mature neurons (Nayak and Herzog, 2010; Wong et al., 2006).

We show that intrathecal KCC2 gene transfer is highly efficient at restoring Cl^- homeostasis in both spinal dorsal horn and DRG neurons and produces complete and long-lasting relief of neuropathic pain. Strikingly, KCC2 gene transfer normalizes, at both pre- and postsynaptic sites, spinal NMDAR activity increased by nerve injury. Our study provides direct evidence that disrupted neuronal Cl^- homeostasis plays a critical role in potentiated synaptic NMDAR activity in neuropathic pain. This information