may be developed to effectively treat neurological diseases, particularly those caused by cellular dysfunction or tissue injury.

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Interneurons Scratch an Itch

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Itch is immensely frustrating. Most studies focus on the cause of itch. In this issue of *Neuron*, Kardon et al. (2014) find that itch can be modulated by inhibitory neurons that produce dynorphin, an endogenous agonist of κ-opioid receptors.

Scientific investigations can be likened to scratch tickets. While individual experiments that we perform may not pan out, the study by Kardon et al. (2014) in this issue of *Neuron* is a winner. These investigators characterize a population of spinal inhibitory neurons and demonstrate that dynorphin, released from these neurons, is a backscratcher responsible for modulating itch (Figure 1).

Itch, also referred to as pruritus, may have evolved as a protective mechanism against threats from arthropods, but it is a prominent feature of inflammatory skin disease and ruins the lives of patients with chronic renal failure, liver disease, and certain malignancies. Its impact on quality of life is comparable to that of pain (Kini et al., 2011). It has been suggested that itch be considered a disease (Yosipovitch, 2011). Drugs with itch as an approved indication are limited to antihistamines and topical steroids and have limited effectiveness. It is recognized that neuromodulators can be remarkably effective in treating some itches and that scratching may provide temporary relief and feel pleasurable, suggesting that complex neurocircuitry and neuromodulatory mechanisms are involved. Accordingly, endogenous molecules may have the potential to reduce the sensation of itch.

Understanding the underlying mechanisms of itch is an intense focus of investigation. Recent advances include the identification of a series of itch-related ligands and receptors as well as peripheral neurons and spinal afferents specialized in transmitting this sensation and distinguishing it from pain (Han et al., 2013; Mishra and Hoon, 2013). It is recognized that itch and pain are part of a complex family dynamic. A few examples are scratching, which alleviates itch but is a noxious stimulus, μ-opioids, which relieve pain but induce itch, and the inhibition of glutaminergic transmission from nociceptors, which reduces pain but increases scratching (Lagerström et al., 2010; Liu et al., 2010).

A key role in sensory processes has been suggested for inhibitory circuits in the spinal cord, consistent with the

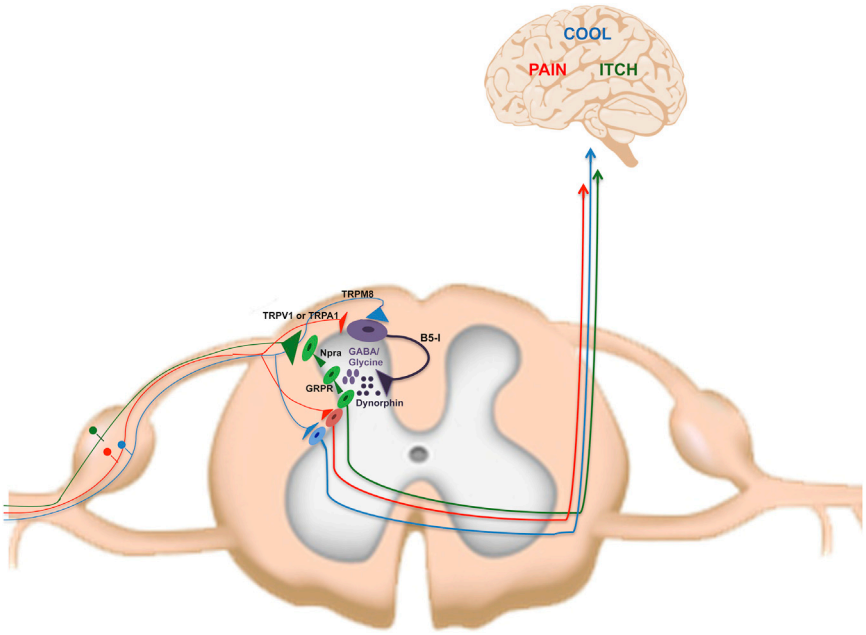

Figure 1. Interneurons that Release Dynorphin Modulate Itch
Neurons that respond to itch, cold, and noxious stimuli innervate and are modulated by B5-I interneurons that produce dynorphin. Capsaicin, mustard oil, and menthol activate TRPV1, TRPA1, and TRPM8, respectively. B5-I, inhibitory neurons that are a focus of the report to which this Preview is directed. GRPR, gastrin-releasing peptide receptor. Npra, natriuretic peptide receptor A is the receptor for the B-type natriuretic peptide BNP, also known as Nppb. Image modified and reproduced under CC-BY license: http://creativecommons.org/licenses/by/3.0/us/legalcode.

relationship between itch and pain. Deletion of the neuronal-specific transcription factor basic helix-loop-helix protein 5 (*Bhlhb5*) resulted in the loss of a subset of inhibitory interneurons, termed B5-I neurons, within the dorsal horn (Ross et al., 2010). These mice scratched without provocation and developed skin lesions as a result. They were additionally sensitive to pruritic agents, whereas nociceptive responses to mechanical, thermal, or chemical stimuli remained unaffected.

A hallmark of itch is that it may be alleviated by counterstimuli, consistent with the existence of inhibitory control pathways. The neural mechanisms and neuromodulators underlying this phenomenon have not been identified definitively. The report from Kardon et al. (2014) represents an important step in addressing this knowledge gap. Dynorphin is a κ-opioid neuropeptide expressed in the CNS. Its precursor is preprodynorphin. Dynorphin is stored in large dense-core vesicles and is released from nerve terminals, selectively acting on κ-opioid receptors (KORs) present in the peripheral nervous system and CNS. Activation of KORs, which couple to G_i/G_0, leads to a decrease in synaptic transmission (Knoll and Carlezon, 2010). It was reported previously that galanin-expressing inhibitory interneurons coexpress dynorphin while also being a source of KORs in the dorsal horn (Sardella et al., 2011).

Kardon et al. (2014) find that B5-I neurons comprise a specific neurochemical population of inhibitory interneurons in the dorsal horn that inhibits itch. Using immunostaining, they found that B5-I neurons coexpress dynorphin and the inhibitory neuropeptide galanin. These mice also express another hallmark of inhibitory neurons, the somatostatin receptor SST_{2A}. Mice in which *Bhlhb5* had been knocked out showed almost a complete loss of galanin- and dynorphin-expressing inhibitory interneurons, a marked decrease in SST_{2A}-expressing neurons, but no change in SST_{2A}-negative neurons.

The antipruritic effect of κ-opioid agonists was first identified in the early 1980s (reviewed by Cowan and Gmerek, 1986). These observations from almost three decades ago continue to be supported by the current finding that scratching behavior, induced by a variety of pruritogens, was inhibited after the administration of κ-opioid agonists, especially to $Bhlhb5^{-/-}$ mice. Kardon et al. (2014) hypothesized that the extensive scratching in these knockout mice was due to reduced κ-opioid signaling associated with the loss of dynorphin-producing cells in the spinal cord. A critical question then arises: is the elevated itch in $Bhlhb5^{-/-}$ mice due to the loss of dynorphin, the lack of fast synaptic inhibition, or both? To address this question, Kardon et al. (2014) examined mice in which the dynorphin precursor had been knocked out ($PPD^{-/-}$). $PPD^{-/-}$ mice have not been reported to scratch spontaneously. It might be expected that $PPD^{-/-}$ mice would scratch more than wild-type controls when itch is induced by pruritogens, indicating an essential role for dynorphin in pruritic inhibitory circuits. However, $PPD^{-/-}$ mice show normal levels or scratching when compared to wild-type animals. This result suggests that the abnormal itch phenotype observed in $Bhlhb5^{-/-}$ animals is not completely dependent on dynorphin. This finding suggests that there is a degree of compensation for the loss of dynorphin in $PPD^{-/-}$ mice, but not the loss of spinal neurons that produce dynorphin. Together, these results suggest a role for dynorphin in quelling acute and chronic itch but that fast inhibitory neurotransmitters, such as GABA and/or glycine, participate in the relief of acute itch by scratching.

Neuropathic itch, a type of chronic itch in people, is a particularly frustrating condition. Shingles, caused by reactivation of varicella in dorsal root ganglia, can cause long-lasting and intense neuropathic pain. It is less well-recognized that some victims suffer from an intense itch rather than pain. The treatment of neuropathic pain, and itch, is yielding somewhat to neuromodulators. As $Bhlhb5^{-/-}$ mice scratch chronically, they may provide a model of neuropathic itch. Kardon et al. (2014) examined the capacity of κ-opioid agonists to relieve scratching in these mice. Two structurally unrelated κ-agonists, nalfurafine and U-50,488, were evaluated. Nalfurafine is approved in Japan for the treatment of itch associated

with chronic kidney disease and is currently being evaluated in the U.S. Nalfurafine and U-50,488 were delivered by intraperitoneal injection and reduced the time mice spent biting and/or licking skin lesions. Histamine and nonhistamine scratching, such as that evoked by chloroquine, is mediated by distinct primary afferents. Nalfurafine was known to reduce scratching evoked by histamine, a finding confirmed here. Nalfurafine and U-50,488 also reduced scratching from chloroquine. These results are consistent with a merging of histamine and nonhistamine neural responses. To confirm that the sensation of itch, and not nociceptive pain, was being evaluated, Kardon et al. (2014) employed the cheek model established by Shimada and LaMotte (2008). Capsaicin, which activates TrpV1 channels and is the active ingredient in hot peppers, generates pain and itch responses that can be distinguished in this model. Low doses of nalfurafine inhibited only the scratching response, implying a degree of selectivity with respect to the treatment of itch versus pain.

Intrathecal injection of κ-agonists or antagonists was used by Kardon et al. (2014) to delve into two additional questions. One question relates to the sequential path in which itch information is relayed. Such information is relayed from the periphery to the spinal cord by upstream Nppb-expressing neurons that communicate with downstream GRPR-expressing neurons in the spinal cord. The intrathecal injection of GRP is known to evoke scratching. When Kardon et al. (2014) coinjected GRP and nalfurafine, scratching was reduced. This result places the action of κ-agonists and, by inference, B5-I interneurons, downstream of GRPR neurons. A second question is whether blockade of KOR signaling would increase an itch-associated response. Intrathecal administration of κ-antagonists was used to answer this question in the affirmative as such compounds resulted in an increased behavioral response to chloroquine. These findings demonstrate the capacity of KOR signaling in the spinal cord to modulate itch up or down.

It is recognized that counterstimuli, including noxious stimuli, scratching, and cold, suppress itch. This suppression occurs through the activation of inhibitory pathways within the spinal cord (Bautista et al., 2014). B5-I interneurons are well positioned to be part of this milieu. To examine this issue further, Kardon et al. (2014) asked whether B5-I neurons receive input from primary afferents expressing TRPV1, TRPA1, and TRPM8, channels that are activated by the itch-inhibiting compounds capsaicin, mustard oil, and menthol, respectively. The answer, as tested using neurophysiologic techniques, was in the affirmative, and a behavioral model was used as a complementary approach. Specifically, the topical application of menthol decreased chloroquine-induced scratching behavior in wild-type mice but not in *Bhlhb5*$^{-/-}$ mice. These findings are consistent with the concept that chemical counterstimuli that ameliorate scratching require B5-I neurons.

The findings in this paper provide a lead for additional directions. κ-opioid agonists have been reported to be helpful for certain itches. Why isn't their effect more general? What is the balance between KOR in the periphery and the spinal cord with respect to ameliorating itch? Might there be endogenous inhibitory mediators in addition to dynorphin that are yet not identified? And what is the role of fast synaptic transmission in modulating itch, as suggested by the observation that mice lacking dynorphin still scratch? Addressing these questions will hopefully pave the way to new therapeutics for people suffering from itch.

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