Itch and Its Inhibition by Counter Stimuli

Lindsey M. Snyder and Sarah E. Ross

Contents

Abstract

Recent studies have made significant progress in the knowledge of how itch sensation is processed, especially the molecular identity of neurons involved in itch signaling, both in the dorsal root ganglion and spinal cord. Despite these advances, the organization of these neurons in dorsal spinal cord circuits and how they interact with other somatosensory modalities, such as pain or temperature, remain relatively unexplored. Recent work from our lab and others has begun to shed light on these questions and will be the focus of this chapter. Here we describe the discovery of B5-I neurons, a population of inhibitory interneurons that function to inhibit itch, and review the evidence that these

L.M. Snyder • S.E. Ross (✉)
Departments of Neurobiology and Anesthesiology, The Center for Pain Research, University of Pittsburgh, 200 Lothrop Street, BSTW1465, Pittsburgh, PA 15213, USA
e-mail: saross@pitt.edu

A. Cowan, G. Yosipovitch (eds.), *Pharmacology of Itch*, Handbook of Experimental Pharmacology 226, DOI 10.1007/978-3-662-44605-8_11

neurons mediate the inhibition of itch by counter stimuli. These studies are helping to solve the long-standing question of why itch makes us scratch.

Keywords
Pruritus • Itch • Dynorphin • Kappa opioid • KOR • Galanin • nNOS • Bhlhb5 • Bhlhe22

1 Introduction

Itch is a very distinctive sensation—it's irritating but not really painful, and it is associated with a very strong desire to scratch. Moreover, scratching causes at least transient relief from itch. In this regard, itch is unmistakably different than pain, which elicits the urge to protect the affected tissue rather than scratch it. And yet, itch and pain can be triggered by the very same chemical agents. For instance, in the eye or an open wound, capsaicin—a prototypical algogen—invariably causes pain. But when applied to undamaged skin, capsaicin can cause itch as well as pain. Histamine—a prototypical pruritogen—causes a pure itch sensation when it is released from mast cells during a bout of hives. But when histamine is injected subcutaneously, it causes pain rather than itch. Furthermore, this itch/pain dichotomy is true for almost every agent that has been studied—formalin, serotonin, endothelin-1, SLIGRL, acetylcholine, and prostaglandin-E2—all of these agents can cause either pain or itch depending on the manner in which they are applied (Ross 2011). These observations imply that the primary sensory afferents that convey itch and those that convey pain must express overlapping subsets of receptors for these chemical agents. But if the sensory neurons for pain and those for itch express the same receptors, how does the nervous system distinguish itch from pain?

One attractive model that may help explain how itch is distinguished from pain is the idea that lateral interaction, possibly at the level of the spinal cord, is involved in sharpening sensory acuity. In particular, inhibitory interneurons may provide cross-modal inhibition to help the nervous system decode somatosensory input. Thus, just as a center-surround network of excitation and inhibition sharpens spatial acuity in the visual system, a stimulus-counter-stimulus network of excitation and inhibition may sharpen the modality input in the somatosensory system. According to this model [which is an elaboration of the selectivity theory (McMahon and Koltzenburg 1992)], the selective activation of sensory neurons that are tuned to detect itch would result in itch, whereas the coactivation of a larger subset of primary afferents (e.g., ones that are tuned to detect noxious input) would result in the inhibition of itch (Fig. 1). One of the reasons that this model is attractive is because a simple neural circuit of this type could explain the everyday phenomenon that scratching (and other counter stimuli) inhibits itch.

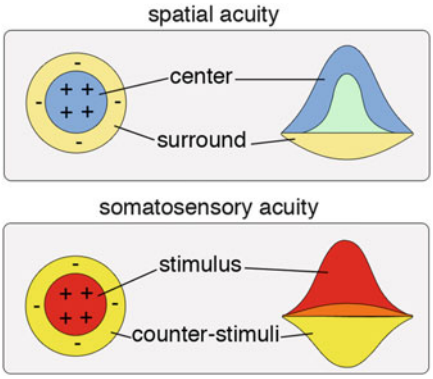

Fig. 1 Sharpening sensory acuity through lateral interaction. In the visual system, a center-surround receptive field sharpens the spatial input for visual acuity. By analogy, a stimulus-counter-stimulus receptive field may sharpen the modality input for somatosensory acuity. In this way, counter stimuli could, for example, inhibit itch

Some indirect evidence for this idea came from two groups that were examining the role of vGLUT2 in somatosensation. These groups made the same fundamental observation, which unexpectedly gave insight into the coding of itch (Lagerstrom et al. 2010; Liu et al. 2010). Glutamate is the fast excitatory neurotransmitter that is released by all primary somatosensory neurons. Although there are three glutamate transporters (vGLUT1–3), many sensory neurons express only vGLUT2. Hence, when vGLUT2 is conditionally removed from primary afferents, the "vGLUT2-only" subset is no longer able to signal via glutamate. Since the vGLUT2-only subset is a large proportion of the C fibers, it was not surprising that the loss of vGLUT2 in primary afferents resulted in blunted pain responses. However, the completely unexpected finding was the vGLUT2-lacking mice scratched all the time and showed elevated itch. These findings imply that glutamate signaling from vGLUT2-only primary afferents normally inhibits itch. However, the neural circuitry underlying this phenomenon remained unclear.

Around this time, strong evidence emerged, suggesting that itch is under inhibitory control at the level of the spinal cord. To study the neural circuits underlying itch, Akiyama et al. (2011) had developed a clever method to record from spinal neurons that are presumably involved in the transmission of itch: they used a model of dry skin itch (to drive ongoing itch) and then recorded from spontaneously active neurons in the spinal cord. Consistent with the idea that counter stimuli inhibit itch, they found that scratching, pinch, and noxious heat inhibited the firing rate of spontaneously active neurons. Moreover, treatment with either strychnine (to block glycinergic inhibition) or bicuculline and saclofen (to block GABAergic inhibition) strongly reduced the scratch-evoked inhibition of these cells. These findings suggested that both GABAergic and glycinergic inhibitory mechanisms in the spinal cord are involved in the inhibition of itch. But the identity of these inhibitory interneurons was not known.

Now, recent work from our lab has revealed that itch is inhibited by a population of spinal interneurons called B5-I neurons, and there is some tantalizing evidence that B5-I may mediate the inhibition of itch by counter stimuli (Kardon et al. 2014;

Ross et al. 2010). Here, we review how these inhibitory interneurons were first discovered and what we know about them.

2 Transcription Factors and the Development of the Dorsal Spinal Cord

Itch, pain, touch, and temperature are first detected in primary sensory afferents that convey this information to the dorsal horn in the spinal cord, particularly laminae I–III. Notably, less than 1 % of the neurons in the dorsal spinal cord are projection neurons that convey somatosensory input to the brain (Todd 2010). The vast majority of neurons in the dorsal horn (~99 %) are local interneurons, a fact which strongly implicates spinal microcircuits in the integration of somatosensory input. Moreover, it is becoming increasingly clear that these neural networks are made of discrete subtypes of spinal interneurons that form stereotyped connections with one another. Importantly, the wiring among these neurons appears to be developmentally programmed by a series of transcription factors. Thus, to understand these local networks, we need to understand the ontogeny of spinal interneurons.

Interneurons within the dorsal horn arise from progenitors that reside in the ventricular zone of the developing spinal cord (Helms and Johnson 2003). These neurons, which are among the last to differentiate, undergo their final round of cell division in between embryonic day 12 and embryonic day 14.5. Two basic types of neurons are born at this time, the so-called dorsal interneurons late A and B (dILA and dILB), which develop into inhibitory and excitatory neurons of the dorsal horn, respectively. Various transcription factors are expressed in neural progenitors and early postmitotic neurons during this time, and these factors are involved in specifying neuronal identity and mediating the differentiation of a neuronal precursor into a specific cell type with stereotyped connectivity.

In particular, the transcription factors of the basic helix-loop-helix (bHLH) and homeodomain factors appear to play key roles in these processes. For instance, inhibitory interneurons in the dorsal horn are not generated in mice lacking the bHLH factor *Ptf1a*, emphasizing the important role of the Ptf1a in mediating inhibitory neuronal fate (Glasgow et al. 2005). Excitatory neurons, in contrast, require the homeodomain factor Tlx3, which is required to suppress the GABAergic fate (Cheng et al. 2004). Both excitatory and inhibitory neurons diversify further during maturation into a large array of distinct neural subtypes (the number of which is not yet known). For instance, various neuropeptides, receptors, and other neuronal markers are expressed by distinct subpopulations of dorsal horn neurons (Polgar et al. 2013b). But, while transcription factors are thought to mediate the terminal differentiation of neurons and their connectivity, the identity of these factors and their specific functions remain poorly understood. Bhlhb5 (also called Bhlhe22) is a bHLH transcription factor that is expressed in the dorsal spinal cord within subsets of dILA and dILB neurons from the time they are postmitotic approximately until P14 (Ross et al. 2010). Thus, based on the

expression pattern of Bhlhb5, we hypothesized that this transcription factor was involved in the terminal differentiation and connectivity of subsets of neurons in the dorsal horn.

3 Mice Lacking the Transcription Factor Bhlhb5 Show Abnormally Elevated Itch

Bhlhb5 is a neural-specific basic helix-loop-helix (bHLH) transcription factor related to the *Drosophila* proneural factor *atonal* (Ross et al. 2003). Bhlhb5 and other closely related family members (namely, Bhlhb4 and Oligs1–3) all function as transcriptional repressors. However, Bhlhb5 is distinct from its family members because Bhlhb5 is selectively expressed in postmitotic neurons rather than in proliferating progenitors. Thus, whereas the Oligs (and likely Bhlhb4) are involved in neuronal fate specification, Bhlhb5 is involved in terminal neuronal differentiation. To investigate the function of Bhlhb5, two independent groups—ours and that of Lin Gan—made *Bhlhb5* knockout mice. These studies revealed an important role for Bhlhb5 in the retina, where it is required for the survival of some amacrine and cone bipolar cells (Feng et al. 2006). In addition, Bhlhb5 is required for the acquisition of area-specific fates in the cortex (Joshi et al. 2008). We found that Bhlhb5 is a transcriptional repressor that uses Prdm8 as an obligate cofactor and that both factors are required for the proper axonal targeting of all cortical projection neurons (Ross et al. 2012). Thus, Bhlhb5 has multiple roles in different regions of the nervous system. However, the most striking phenotype of $Bhlhb5^{-/-}$ mice is that they all develop self-inflicted skin lesions, which prompted us to investigate somatosensation in these mice (Ross et al. 2010).

Initially, it was not clear why $Bhlhb5^{-/-}$ mice, which behave normally until around 4–6 weeks of age, suddenly develop self-inflicted skin lesions. At the time of these studies, only a few animals with skin lesions had been analyzed in detail, and in these cases it was concluded that the mice in question either lacked sensitivity to pain ($Drg11^{-/-}$ mice) or suffered from obsessive-compulsive disorder ($Hoxb8^{-/-}$ mice) (Chen et al. 2001; Greer and Capecchi 2002). Indeed, when we (naively) first analyzed somatosensation in adult $Bhlhb5^{-/-}$ mice (which, importantly, already had skin lesions), we found that these mice showed a very blunted response to noxious input. Based on these findings, we erroneously concluded that the self-injury observed in $Bhlhb5^{-/-}$ mice was due to an absence of pain. Fortunately (and as a testimony to the efficacy of the review process), the reviewers of our manuscript questioned our interpretation and asked us to investigate the possibility of abnormal itch in $Bhlhb5^{-/-}$ mice. When we reevaluated the behavior of $Bhlhb5^{-/-}$ mice, this time analyzing mice before the onset of skin lesions, we realized that our first interpretation was completely wrongheaded. In fact, prior to the onset of skin lesions, $Bhlhb5^{-/-}$ mice show normal responses in most sensory tests including chemical, mechanical, and heat nociception (Ross et al. 2010). However, $Bhlhb5^{-/-}$ mice scratch significantly more than wild-type littermates following the application of all of the itch-inducing agents tested. Therefore, the

lack of Bhlhb5 expression led to an increase in itch sensitivity, but left pain and other somatosensory modalities relatively intact. Furthermore, these findings suggested that the self-inflicted skin lesions in *Bhlhb5*$^{-/-}$ mice were the result of excessive licking and scratching due to elevated itch.

4 Pathological Itch in Bhlhb5$^{-/-}$ Mice Is Due to the Loss of B5-I Neurons

Having determined that *Bhlhb5*$^{-/-}$ mice have abnormally elevated itch, the next step was to identify which cells were responsible for this phenotype. Since Bhlhb5 is expressed in numerous regions of the nervous system, the cellular basis of elevated itch was not completely obvious. Though Bhlhb5 is expressed in subsets of neurons in the dorsal horn (where itch is first integrated), this transcription factor is also expressed in some primary sensory afferents, the brainstem, and many other regions of the brain that might theoretically be involved in the processing of itch. To determine the neurons responsible for the elevated itch in *Bhlhb5*$^{-/-}$ mice, we used a genetic approach to selectively remove *Bhlhb5* from different regions of the nervous system (Ross et al. 2010). Using this conditional ablation strategy, we were able to ask whether deletion of *Bhlhb5* in specific areas of the nervous system was sufficient to recapitulate the phenotype seen in the constitutive *Bhlhb5*$^{-/-}$ mice. Upon loss of *Bhlhb5* from primary afferents, the resulting mice were normal with respect to itch. Likewise, upon loss of *Bhlhb5* from the dorsal telencephalon, the resulting mice had no sensory phenotypes. However, loss of *Bhlhb5* from the spinal cord (using the Hoxb8-cre line) was sufficient for the abnormally elevated itch and the development of skin lesions (unpublished observation). This finding suggested a key role of Bhlhb5 in the spinal cord. Since Bhlhb5 is expressed in both excitatory and inhibitory neurons within the spinal cord, we used cre lines that caused selective removal of *Bhlhb5* in excitatory and inhibitory neurons, respectively, to determine which type of spinal neurons were involved. These experiments revealed that loss of *Bhlhb5* within excitatory neurons of the dorsal horn (using Tlx3-cre) had no effect on itch sensitivity, whereas loss of *Bhlhb5* within inhibitory neurons (using Pax2-cre) was sufficient for abnormally elevated itch. Together, these experiments revealed that Bhlhb5 is required in inhibitory spinal interneurons for normal itch.

In newborn mice, Bhlhb5 is expressed in 7 % of neurons within the dorsal spinal cord. Of these, approximately one quarter of Bhlhb5-expressing neurons are excitatory and three quarters are inhibitory. At the time, there were no other markers for the Bhlhb5-expressing cells, and so there was no way to see what happened to the Bhlhb5-expressing cells in the absence of *Bhlhb5*. To resolve this problem, we used another genetic approach to permanently label all the cells that had ever expressed *Bhlhb5*. Specifically, we generated a *Bhlhb5-cre* knockin allele, which we then crossed with cre-responsive reporters (Ross et al. 2010). Using this approach, we discovered that Bhlhb5 is required for the survival of Bhlhb5-expressing neurons in the spinal cord. Without it, many of the neurons that should have expressed Bhlhb5

were missing. This discovery implied that the loss of a specific population of inhibitory interneurons during development results in abnormal itch, and we called these spinal interneurons B5-I neurons, since they are the ***I***nhibitory subset of Bhlh***b5***-expressing neurons.

5 B5-I Neurons Function to Inhibit Itch

Although we had identified the neurons responsible for abnormal itch, we still didn't know very much about them. To study these cells in greater detail, we began collaborating with Andrew Todd. His previous work had revealed that spinal inhibitory neurons can be divided into subgroups based on the expression of neurochemical markers (Polgar et al. 2013b). In particular, approximately one half of the inhibitory neurons in laminae I and II of the spinal cord express somatostatin receptor sst_{2A}, and B5-I neurons were found to belong to this subset (Kardon et al. 2014). The discovery that all B5-I neurons express sst_{2A} (which inhibits neurons) was important because it provided us with the tools that we needed to address a key question. Specifically, hitherto it was still not clear whether B5-I neurons function in the adult animal to inhibit itch or whether the survival of B5-I neurons was critical for the establishment of proper itch circuits—in other words, we could not distinguish between an adult function and a developmental role for B5-I neurons. Fortuitously, the finding that B5-I neurons express sst_{2A} provided an opportunity to directly test whether B5-I neurons inhibit itch in adult mice. Specifically, we reasoned that if B5-I neurons suppress itch, then inhibition of B5-I neurons through activation of sst_{2A} would result in spontaneous itch. In keeping with this idea, we found that intrathecal injection of the sst_{2A} agonist, octreotide, resulted in spontaneous scratching behavior. Moreover, this effect was lost in $Bhlhb5^{-/-}$ mice that are lacking B5-I neurons (Fig. 2). These data revealed that disinhibition of B5-I neurons causes itch, indicating that B5-I neurons normally function to inhibit itch.

6 B5-I Neurons Release the Kappa Opioid Dynorphin and Kappa Agonists Inhibit Itch

The inhibitory interneurons that express sst_{2A} are not a single population, and previous work by Andrew Todd had shown these interneurons can be further subdivided based on the expression of distinct neurochemical markers (Polgar et al. 2013a; Spike et al. 1998). When we looked at which of these subpopulations made up B5-I neurons, we discovered that B5-I neurons are composed of two mostly nonoverlapping subpopulations, one expressing the neuropeptide galanin and one expressing neuronal nitric oxide synthase (nNOS). Moreover, both galanin-expressing and nNOS-expressing subpopulations were almost completely missing in $Bhlhb5^{-/-}$ mice, whereas other populations of inhibitory neurons were unaltered (Kardon et al. 2014).

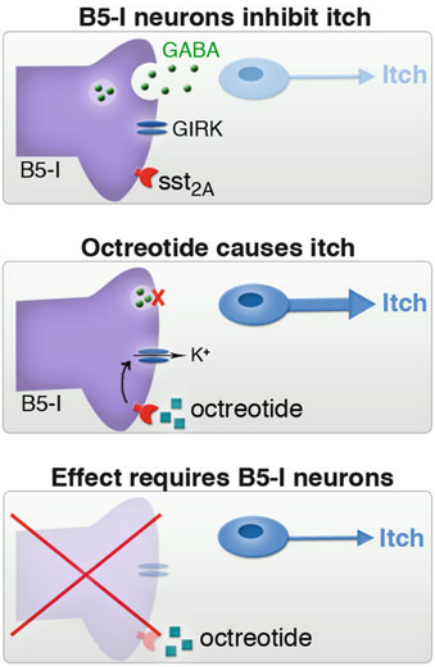

Fig. 2 Evidence that B5-I neurons function to inhibit itch. (**a**) Normally, itch is inhibited by B5-I neurons. (**b**) Activation of the somatostatin receptor sst_{2A} with octreotide results in the inhibition of B5-I neurons and spontaneous itch. (**c**) Octreotide has no effect in $Bhlhb5^{-/-}$ mice, which are lacking B5-I neurons

This discovery raised the important question of whether one or both of these subpopulations are involved in the inhibition of itch. Although we still don't know the answer to this question with certainty, we were particularly interested in the galanin subpopulation because previous work had shown that galanin-expressing interneurons also express the endogenous kappa opioid, dynorphin (Sardella et al. 2011). The idea that the release of dynorphin from B5-I neurons could be a potential mechanism through which they inhibit itch sensation was an attractive idea since there was already some precedent for the idea that kappa opioids inhibit itch (Inan and Cowan 2004; Ko et al. 2003; Togashi et al. 2002). Consistent with these previous studies, we found that pretreatment with kappa opioid agonists significantly decreased scratching in response to both histamine-dependent and histamine-independent pruritogens in wild-type mice (Kardon et al. 2014). Kappa opioid agonists also significantly reduced the elevated scratching response observed in $Bhlhb5^{-/-}$ mice, consistent with the idea that it is the absence of dynorphin in $Bhlhb5^{-/-}$ mice that is partially responsible for their elevated itch.

These findings raised the questions as to whether the GABA/glycine-mediated inhibition provided by B5-I galanin and nNOS cells was responsible for the elevated itch in $Bhlhb5^{-/-}$ mice or whether it was due solely to the decrease of dynorphin signaling. To evaluate this question, we analyzed the preprodynorphin knockout mice ($PPD^{-/-}$), which are missing dynorphin but not spinal dynorphin-expressing neurons. We found that loss of $PPD^{-/-}$ had no effect on itch sensitivity, and $PPD^{-/-}$ mice do not develop self-inflicted skin lesions (Kardon et al. 2014).

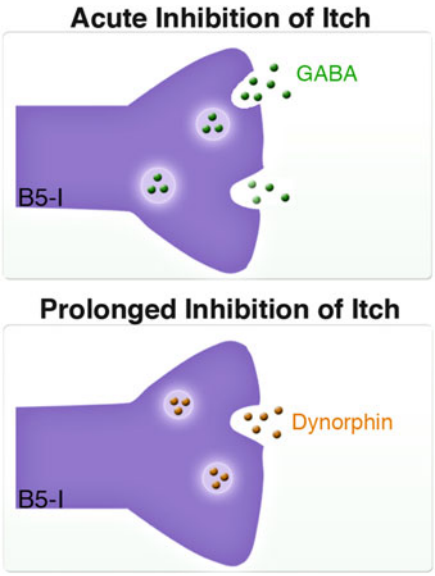

Fig. 3 Model for the acute and prolonged inhibition of itch by B5-I neurons. Fast-acting inhibitory neurotransmitters such as GABA may provide instantaneous relief from itch. The inhibitory neuromodulator dynorphin may provide sustained relief from itch, lasting minutes to hours

These observations point to a key difference between the loss of a neuropeptide and a loss of a neuronal subtype. Thus, whereas compensatory mechanisms may be able to atone for the loss of dynorphin, they cannot fully compensate for the loss of dynorphin-releasing neurons in *Bhlhb5*$^{-/-}$ mice. In conjunction, this evidence points to a role for both GABA/glycine- and kappa opioid-mediated inhibition in the regulation of itch sensation. One possibility is that GABA and glycine, which are fast-acting neurotransmitters, are involved in the immediate relief of itch that is felt upon scratching, whereas dynorphin, which is a neuromodulator that signals via Gi/o-coupled signaling cascades, is involved in the prolonged inhibition of itch (Fig. 3).

The identity of the cells expressing the kappa opioid receptor (KOR) is of particular interest, as they are likely to be the neurons that are inhibited by dynorphin and hence are likely intimately involved in the processing of itch sensation in the dorsal horn. Previous studies have established a key role for gastrin-releasing-peptide (GRP) receptor expressing excitatory interneurons in the transmission of itch signals within the dorsal horn (Sun et al. 2009; Mishra and Hoon 2013), and so we wondered whether these neurons might express KOR. We found that pretreatment with a kappa agonist significantly reduced scratching in response to GRP application (Kardon et al. 2014). This finding suggests that B5-I neurons could possibly target GRPR neurons directly. On the other hand, it is possible that dynorphin targets other interneurons downstream of the GRPR neurons. In either case, identifying the spinal neurons that express KOR is important since they will likely have an important role in the integration of itch.

7 Kappa Agonists as a Therapeutic Agent for Chronic Pruritus

Given the huge number of people worldwide that suffer from chronic itch, it is important to consider the therapeutic potential of kappa opioid agonists. To address this question, we used *Bhlhb5*$^{-/-}$ mice that have skin lesions as a model of neuropathic itch. Importantly, treatment of these mice with kappa opioid agonists significantly decreased time spent scratching, supporting the idea that kappa opioid agonists may serve as potentially effective clinical treatments (Kardon et al. 2014). Indeed, several clinical trials support the feasibility of kappa opioid agonists to treat chronic pruritus (Kumagai et al. 2012; Wikstrom et al. 2005). More recently, the neuropathic itch in *Bhlhb5*$^{-/-}$ mice has also been resolved by transplanting GABAergic neurons into the spinal cord. Thus, it appears that either increased GABAergic tone in the spinal cord or increased KOR signaling can reduce itch.

7.1 Kappa Agonists Are Selective for Itch

The kappa opioid receptor is closely related to the mu and delta opioid receptors. When the kappa opioid receptor was first discovered, there was great enthusiasm in the pharmaceutical industry for the idea that kappa opioids might offer relief from pain without the addiction and potential for abuse, which are observed with mu agonists like morphine. Numerous kappa agonists were developed and many were tested in clinical trials. Unfortunately, kappa agonists turned out to be poor at blocking pain, and although there are hundreds of papers testing the role of kappa agonists in animal models, only one agonist (U50,488) was ever tested in the KOR$^{-/-}$ mouse to confirm specificity of the agonist. Furthermore, even in this case, the dose of U50,488 that results in decreased nociceptive responses is one that appears to have sedative effects in the mouse (Simonin et al. 1998). Thus, the degree to which kappa opioid signaling truly inhibits pain remains an open question. This issue prompted us to analyze the degree to which the kappa agonists used on our study were selective for pain vs. itch. To address this question, we turned to the cheek model of itch developed by Lamotte's group (Shimada and LaMotte 2008). In this model, itch and pain behaviors can be distinguished in the same assay by quantifying scratching vs. wiping responses. Agents that cause pain result in wiping with the forepaw, whereas agents that cause itch result in scratching with the hindpaw. Using this model, we found that the kappa agonist nalfurafine significantly reduced chloroquine-induced scratching but had no effect on capsaicin-induced wiping (Kardon et al. 2014). Thus, at least under some conditions, kappa agonists selectively inhibit itch but not pain.

8 Increasing Evidence That Opioid Receptor Subtypes Show Modality Selectivity

An exciting idea that emerges from these experiments is the possibility that different opioid subtypes are involved in regulating different aspects of somatosensation. Mu opioid agonists, such as morphine, are among the most widely used for the treatment of pain. However, it has been reported that epidural morphine administration in patients often resulted in pruritus (Kjellberg and Tramer 2001). This side effect, however, is reduced by treatment with the drug nalbuphine, which acts as a kappa opioid agonist and mu opioid antagonist (Liao et al. 2011). Thus, nalbuphine may reduce itch, at least in part, through its action at the kappa opioid receptor. Consistent with this idea is the report of kappa opioid agonists reducing morphine-induced itch in monkeys (Ko et al. 2003). Considering these data, it is possible that mu and kappa opioids play distinct, opposing roles in somatosensory modulation in which mu opioids decrease pain and kappa opioids decrease itch. Intriguingly, studies of the delta opioid receptor are now suggesting that delta agonists are likewise specific to certain modalities of somatosensation. In particular, the delta opioid receptor is specifically expressed in mechanically sensitive primary afferents, and delta agonists specifically reduce mechanical pain (Bardoni et al. 2014; Scherrer et al. 2009). Thus, mu agonists may target heat pain, delta agonists may reduce mechanical pain, and kappa agonists may selectively inhibit itch. The idea that distinct opioid subtypes may differentially modulate neural function is in keeping with what is observed in other regions of the nervous system where, for example, mu and kappa opioids have opposing effects on body-temperature regulation in the hypothalamus (Rawls and Benamar 2011) and on emotional state in the limbic system (Schlaepfer et al. 1998).

9 Inhibition of Itch by Counter Stimuli

Several studies in humans have reported that both noxious hot and cold can decrease reported itch sensation following the application of histamine to the skin (Ward et al. 1996; Yosipovitch et al. 2007). Previous work has shown that glycine and GABA activity within the dorsal horn is important for the decrease in pruritogen-evoked activity of neurons within the dorsal horn caused by counter stimuli (Akiyama et al. 2011). This finding suggests that inhibitory interneurons mediate the inhibition of itch by different sensory modalities such as scratching, noxious input, heat, and cool (Ma 2010; Patel and Dong 2010; Ross 2011). Since B5-I neurons function to inhibit itch, we reasoned that these neurons might be the neural substrate that mediates the inhibition of itch by counter stimuli. To test this idea, we performed a couple of different types of experiments to address whether B5-I neurons might be involved in this type of inhibition. Here we describe the electrophysiological and behavioral experiments that suggest that B5-I neurons may mediate the inhibition of itch by chemical counter stimuli.

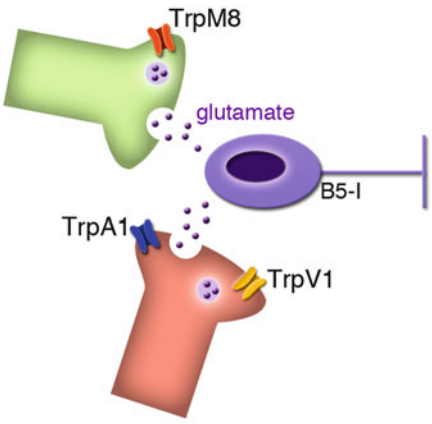

Fig. 4 TRPM8-, TRPA1-, and TRPV1-expressing sensory neurons directly target B5-I neurons. Depolarization of primary afferents by central application of menthol, mustard oil, or capsaicin causes glutamate release onto B5-I neurons

Chemicals such as capsaicin, mustard oil, and menthol are included in creams used to treat itch (Patel and Yosipovitch 2010). These chemicals are agonists for cation channels TRPV1, TRPA1, and TRPM8, respectively, which are expressed on primary afferent nerves. We therefore reasoned that if B5-I neurons mediate the inhibition of itch by these chemical counter stimuli, then B5-I neurons ought to receive input from these primary afferent fibers. To test this idea, we took advantage of the fact that application of these chemicals to an in vitro spinal cord preparation activates the primary afferent terminals, causing the release of glutamate and subsequent activation of postsynaptic neurons in the spinal cord. In order to determine if B5-I neurons receive input from these classes of primary afferents, electrophysiological recordings of B5-I neuron activity were performed during the application of each of these three chemicals. Consistent with our hypothesis, we observed a significant increase in the frequency of excitatory postsynaptic currents (EPSCs) following the application of each chemical (Kardon et al. 2014). Furthermore, this increase in EPSC frequency was maintained in the presence of tetrodotoxin (TTX), which blocks action potential propagation. These results suggest that B5-I neurons receive direct synaptic input from primary afferent terminals expressing TRPV1, TRPA1, and TRPM8 channels (Fig. 4).

To more rigorously test the idea that B5-I neurons mediate the inhibition of itch by counter stimuli, we developed a behavior model of the inhibition of itch by menthol. Prior studies in humans had shown that topical application of menthol reduces itch intensity, and menthol is commonly used in topical antipruritic treatments (Bromm et al. 1995; Patel et al. 2007). We found that, just as is observed in humans, topical application of an 8 % menthol-containing solution significantly reduced pruritogen-induced itch in mice. Next we reasoned that if B5-I neurons mediate the inhibition of itch by menthol, then menthol should not inhibit itch in mice that lack B5-I neurons. Consistent with this idea, we found that although menthol reduces itch in wild-type mice, it did not do so in *Bhlhb5*$^{-/-}$ mice, which lack B5-I neurons. These data suggest that B5-I neurons are necessary for mediating the inhibition of itch by the counter stimulus, menthol (Fig. 5).

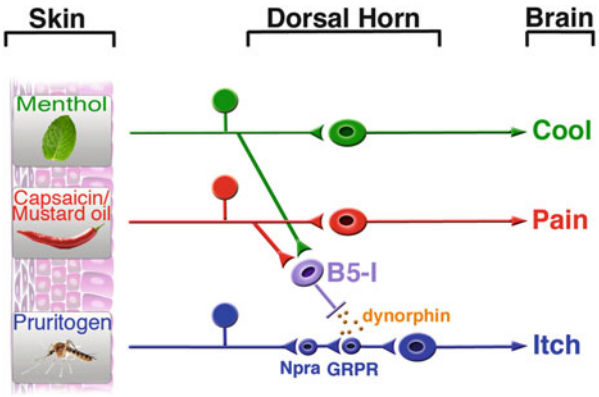

Fig. 5 B5-I neurons may mediate the inhibition of itch by the counter stimuli menthol, capsaicin, and mustard oil. Mice lacking B5-I neurons no longer exhibit the inhibition of itch by counter stimuli

While B5-I neurons seem selective for the inhibition of itch, it is possible that other inhibitory interneuron populations in the spinal cord are involved in modulating other somatosensory modalities. A recent paper suggests that a population of inhibitory interneurons mediates the heat inhibition of cold sensation (McCoy et al. 2013). They propose this model in light of the finding that ablating primary afferents containing CGRPα reduced heat and itch sensitivity but increased cold sensitivity. Although the identity of these interneurons remains to be determined, it is possible that these and other subsets of interneurons within the dorsal horn play similar roles as B5-I neurons but mediate different sensations.

10 Conclusions

Though the specific circuitry underlying the processing of itch sensation in the dorsal spinal cord remains largely uncharacterized, significant advances are being made, describing populations of interneurons and outlining their importance in somatosensory processing (Kardon et al. 2014; Mishra and Hoon 2013; Ross et al. 2010; Sun et al. 2009; Wang et al. 2013; Xu et al. 2013). Discovering the molecular identity of these neurons is important, as it gives us the opportunity to identify and manipulate these populations. Using molecular genetic tools combined with other techniques allows us to probe the circuitry underlying itch sensation. Further understanding of the properties and connections of neurons involved in itch sensation can potentially lead to the development of more effective pharmacological treatments for pruritic conditions.

Acknowledgments We thank Stephanie Buerk for the help with figures as well as Nathan Vogler and Brittany Humensky for the suggestions.

References

Akiyama T, Iodi Carstens M, Carstens E (2011) Transmitters and pathways mediating inhibition of spinal itch-signaling neurons by scratching and other counterstimuli. PLoS One 6(7):e22665. doi:10.1371/journal.pone.0022665

Bardoni R, Tawfik VL, Wang D, Francois A, Solorzano C, Shuster SA, Choudhury P, Betelli C, Cassidy C, Smith K, de Nooij JC, Mennicken F, O'Donnell D, Kieffer BL, Woodbury CJ, Basbaum AI, MacDermott AB, Scherrer G (2014) Delta opioid receptors presynaptically regulate cutaneous mechanosensory neuron input to the spinal cord dorsal horn. Neuron 81 (6):1312–1327. doi:10.1016/j.neuron.2014.01.044

Bromm B, Scharein E, Darsow U, Ring J (1995) Effects of menthol and cold on histamine-induced itch and skin reactions in man. Neurosci Lett 187(3):157–160

Chen ZF, Rebelo S, White F, Malmberg AB, Baba H, Lima D, Woolf CJ, Basbaum AI, Anderson DJ (2001) The paired homeodomain protein DRG11 is required for the projection of cutaneous sensory afferent fibers to the dorsal spinal cord. Neuron 31(1):59–73

Cheng L, Arata A, Mizuguchi R, Qian Y, Karunaratne A, Gray PA, Arata S, Shirasawa S, Bouchard M, Luo P, Chen CL, Busslinger M, Goulding M, Onimaru H, Ma Q (2004) Tlx3 and Tlx1 are post-mitotic selector genes determining glutamatergic over GABAergic cell fates. Nat Neurosci 7(5):510–517. doi:10.1038/nn1221

Feng L, Xie X, Joshi PS, Yang Z, Shibasaki K, Chow RL, Gan L (2006) Requirement for Bhlhb5 in the specification of amacrine and cone bipolar subtypes in mouse retina. Development 133 (24):4815–4825. doi:10.1242/dev.02664

Glasgow SM, Henke RM, Macdonald RJ, Wright CV, Johnson JE (2005) Ptf1a determines GABAergic over glutamatergic neuronal cell fate in the spinal cord dorsal horn. Development 132(24):5461–5469. doi:10.1242/dev.02167

Greer JM, Capecchi MR (2002) Hoxb8 is required for normal grooming behavior in mice. Neuron 33(1):23–34

Helms AW, Johnson JE (2003) Specification of dorsal spinal cord interneurons. Curr Opin Neurobiol 13(1):42–49

Inan S, Cowan A (2004) Kappa opioid agonists suppress chloroquine-induced scratching in mice. Eur J Pharmacol 502(3):233–237. doi:10.1016/j.ejphar.2004.09.010

Joshi PS, Molyneaux BJ, Feng L, Xie X, Macklis JD, Gan L (2008) Bhlhb5 regulates the postmitotic acquisition of area identities in layers II-V of the developing neocortex. Neuron 60(2):258–272. doi:10.1016/j.neuron.2008.08.006

Kardon AP, Polgar E, Hachisuka J, Snyder LM, Cameron D, Savage S, Cai X, Karnup S, Fan CR, Hemenway GM, Bernard CS, Schwartz ES, Nagase H, Schwarzer C, Watanabe M, Furuta T, Kaneko T, Koerber HR, Todd AJ, Ross SE (2014) Dynorphin acts as a neuromodulator to inhibit itch in the dorsal horn of the spinal cord. Neuron 82(3):573–586. doi:10.1016/j.neuron.2014.02.046

Kjellberg F, Tramer MR (2001) Pharmacological control of opioid-induced pruritus: a quantitative systematic review of randomized trials. Eur J Anaesthesiol 18(6):346–357

Ko MC, Lee H, Song MS, Sobczyk-Kojiro K, Mosberg HI, Kishioka S, Woods JH, Naughton NN (2003) Activation of kappa-opioid receptors inhibits pruritus evoked by subcutaneous or intrathecal administration of morphine in monkeys. J Pharmacol Exp Ther 305(1):173–179. doi:10.1124/jpet.102.044909

Kumagai H, Ebata T, Takamori K, Miyasato K, Muramatsu T, Nakamoto H, Kurihara M, Yanagita T, Suzuki H (2012) Efficacy and safety of a novel k-agonist for managing intractable pruritus in dialysis patients. Am J Nephrol 36(2):175–183. doi:10.1159/000341268

Lagerstrom MC, Rogoz K, Abrahamsen B, Persson E, Reinius B, Nordenankar K, Olund C, Smith C, Mendez JA, Chen ZF, Wood JN, Wallen-Mackenzie A, Kullander K (2010) VGLUT2-dependent sensory neurons in the TRPV1 population regulate pain and itch. Neuron 68(3):529–542. doi:10.1016/j.neuron.2010.09.016

Liao CC, Chang CS, Tseng CH, Sheen MJ, Tsai SC, Chang YL, Wong SY (2011) Efficacy of intramuscular nalbuphine versus diphenhydramine for the prevention of epidural morphine-induced pruritus after cesarean delivery. Chang Gung Med J 34(2):172–178

Liu Y, Abdel Samad O, Zhang L, Duan B, Tong Q, Lopes C, Ji RR, Lowell BB, Ma Q (2010) VGLUT2-dependent glutamate release from nociceptors is required to sense pain and suppress itch. Neuron 68(3):543–556. doi:10.1016/j.neuron.2010.09.008

Ma Q (2010) Labeled lines meet and talk: population coding of somatic sensations. J Clin Invest 120(11):3773–3778. doi:10.1172/JCI43426

McCoy ES, Taylor-Blake B, Street SE, Pribisko AL, Zheng J, Zylka MJ (2013) Peptidergic CGRPalpha primary sensory neurons encode heat and itch and tonically suppress sensitivity to cold. Neuron 78(1):138–151. doi:10.1016/j.neuron.2013.01.030

McMahon SB, Koltzenburg M (1992) Itching for an explanation. Trends Neurosci 15(12):497–501

Mishra SK, Hoon MA (2013) The cells and circuitry for itch responses in mice. Science 340 (6135):968–971. doi:10.1126/science.1233765

Patel KN, Dong X (2010) An itch to be scratched. Neuron 68(3):334–339. doi:10.1016/j.neuron.2010.10.018

Patel T, Ishiuji Y, Yosipovitch G (2007) Menthol: a refreshing look at this ancient compound. J Am Acad Dermatol 57(5):873–878. doi:10.1016/j.jaad.2007.04.008

Patel T, Yosipovitch G (2010) Therapy of pruritus. Expert Opin Pharmacother 11(10):1673–1682. doi:10.1517/14656566.2010.484420

Polgar E, Durrieux C, Hughes DI, Todd AJ (2013a) A quantitative study of inhibitory interneurons in laminae I-III of the mouse spinal dorsal horn. PLoS One 8(10):e78309. doi:10.1371/journal.pone.0078309

Polgar E, Sardella TC, Tiong SY, Locke S, Watanabe M, Todd AJ (2013b) Functional differences between neurochemically defined populations of inhibitory interneurons in the rat spinal dorsal horn. Pain 154(12):2606–2615. doi:10.1016/j.pain.2013.05.001

Rawls SM, Benamar K (2011) Effects of opioids, cannabinoids, and vanilloids on body temperature. Front Biosci (Schol Ed) 3:822–845

Ross SE (2011) Pain and itch: insights into the neural circuits of aversive somatosensation in health and disease. Curr Opin Neurobiol 21(6):880–887. doi:10.1016/j.conb.2011.10.012

Ross SE, Greenberg ME, Stiles CD (2003) Basic helix-loop-helix factors in cortical development. Neuron 39(1):13–25

Ross SE, Mardinly AR, McCord AE, Zurawski J, Cohen S, Jung C, Hu L, Mok SI, Shah A, Savner EM, Tolias C, Corfas R, Chen S, Inquimbert P, Xu Y, McInnes RR, Rice FL, Corfas G, Ma Q, Woolf CJ, Greenberg ME (2010) Loss of inhibitory interneurons in the dorsal spinal cord and elevated itch in Bhlhb5 mutant mice. Neuron 65(6):886–898. doi:10.1016/j.neuron.2010.02.025

Ross SE, McCord AE, Jung C, Atan D, Mok SI, Hemberg M, Kim TK, Salogiannis J, Hu L, Cohen S, Lin Y, Harrar D, McInnes RR, Greenberg ME (2012) Bhlhb5 and Prdm8 form a repressor complex involved in neuronal circuit assembly. Neuron 73(2):292–303. doi:10.1016/j.neuron.2011.09.035

Sardella TC, Polgar E, Garzillo F, Furuta T, Kaneko T, Watanabe M, Todd AJ (2011) Dynorphin is expressed primarily by GABAergic neurons that contain galanin in the rat dorsal horn. Mol Pain 7:76. doi:10.1186/1744-8069-7-76

Scherrer G, Imamachi N, Cao YQ, Contet C, Mennicken F, O'Donnell D, Kieffer BL, Basbaum AI (2009) Dissociation of the opioid receptor mechanisms that control mechanical and heat pain. Cell 137(6):1148–1159. doi:10.1016/j.cell.2009.04.019

Schlaepfer TE, Strain EC, Greenberg BD, Preston KL, Lancaster E, Bigelow GE, Barta PE, Pearlson GD (1998) Site of opioid action in the human brain: mu and kappa agonists' subjective and cerebral blood flow effects. Am J Psychiatry 155(4):470–473

Shimada SG, LaMotte RH (2008) Behavioral differentiation between itch and pain in mouse. Pain 139(3):681–687. doi:10.1016/j.pain.2008.08.002

Simonin F, Valverde O, Smadja C, Slowe S, Kitchen I, Dierich A, Le Meur M, Roques BP, Maldonado R, Kieffer BL (1998) Disruption of the kappa-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective kappa-agonist U-50,488 H and attenuates morphine withdrawal. EMBO J 17(4):886–897. doi:10.1093/emboj/17.4.886

Spike RC, Kerr R, Maxwell DJ, Todd AJ (1998) GluR1 and GluR2/3 subunits of the AMPA-type glutamate receptor are associated with particular types of neurone in laminae I-III of the spinal dorsal horn of the rat. Eur J Neurosci 10(1):324–333

Sun YG, Zhao ZQ, Meng XL, Yin J, Liu XY, Chen ZF (2009) Cellular basis of itch sensation. Science 325(5947):1531–1534. doi:10.1126/science.1174868

Todd AJ (2010) Neuronal circuitry for pain processing in the dorsal horn. Nat Rev Neurosci 11 (12):823–836. doi:10.1038/nrn2947

Togashi Y, Umeuchi H, Okano K, Ando N, Yoshizawa Y, Honda T, Kawamura K, Endoh T, Utsumi J, Kamei J, Tanaka T, Nagase H (2002) Antipruritic activity of the kappa-opioid receptor agonist, TRK-820. Eur J Pharmacol 435(2–3):259–264

Wang X, Zhang J, Eberhart D, Urban R, Meda K, Solorzano C, Yamanaka H, Rice D, Basbaum AI (2013) Excitatory superficial dorsal horn interneurons are functionally heterogeneous and required for the full behavioral expression of pain and itch. Neuron 78(2):312–324. doi:10.1016/j.neuron.2013.03.001

Ward L, Wright E, McMahon SB (1996) A comparison of the effects of noxious and innocuous counterstimuli on experimentally induced itch and pain. Pain 64(1):129–138

Wikstrom B, Gellert R, Ladefoged SD, Danda Y, Akai M, Ide K, Ogasawara M, Kawashima Y, Ueno K, Mori A, Ueno Y (2005) Kappa-opioid system in uremic pruritus: multicenter, randomized, double-blind, placebo-controlled clinical studies. J Am Soc Nephrol 16 (12):3742–3747. doi:10.1681/ASN.2005020152

Xu Y, Lopes C, Wende H, Guo Z, Cheng L, Birchmeier C, Ma Q (2013) Ontogeny of excitatory spinal neurons processing distinct somatic sensory modalities. J Neurosci 33 (37):14738–14748. doi:10.1523/JNEUROSCI. 5512-12.2013

Yosipovitch G, Duque MI, Fast K, Dawn AG, Coghill RC (2007) Scratching and noxious heat stimuli inhibit itch in humans: a psychophysical study. Br J Dermatol 156(4):629–634. doi:10.1111/j.1365-2133.2006.07711.x