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Transmission of Pruriceptive Signals

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Abstract

In this chapter we discuss the many recent discoveries of the mechanisms by which itch is transmitted: the neurotransmitters and the responses they trigger, the mechanisms by which specific neuronal targets are activated, and the specificity of the pathways. Current data reveal that DRG-neurons and spinal cord cells use a remarkably selective set of transmitters to convey pruritic information from the periphery to the brain: glutamate and Nppb are released from primary itch-sensory cells; these molecules activate secondary spinal cord pruriceptive specific neurons, which in turn utilize Grp to activate tertiary pruriceptive selective neurons. Intersecting this basic linear excitatory pathway, inhibitory input from dynorphin and neurons that express the somatostatin receptor modify itch-sensation. Cumulatively, these studies paint an elegantly simple picture of how itch signals are transformed and integrated in the spinal cord, and open new avenues for research efforts aimed at understanding and better treating itch.

Keywords

Pruritic; itch; neurotransmitters; receptors; Nppb; Grp; Grpr; dynorphin; somatostatin

1. Transmission of itch signals from primary itch-receptor neurons

Historically, it was thought that, like all other classes of somatosensory neurons, the excitatory transmitter glutamate was the primary neurotransmitter used by pruritic sensory neurons. More recently, the number of molecules reported to be involved in the initial step of itch transmission has expanded to include gastrin releasing peptide (Grp), natriuretic polypeptide b (Nppb), and neuromedin b (Nmb) (Koga et al, 2011; Mishra et al, 2012; Mishra & Hoon, 2013; Sukhtankar & Ko, 2013; Sun & Chen, 2007; Sun et al, 2009).

The role for a potential glutamate co-transmitter released from primary itch-neurons is supported by studies in which mice were engineered to lose glutamate transmission from selective subsets of somatosensory neurons. This was accomplished by eliminating the gene responsible for the uptake of glutamate into synaptic vesicles (vesicular glutamate transporter 2; VGLUT2) in TRPV1 or Nav1.8-neurons. The resultant mutant animals exhibit spontaneous itch-behavior (Lagerstrom et al, 2010; Liu et al, 2010). This result suggests that in the absence of glutamate, some other transmitter is used at the first itch synapse (and that

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glutamate mediated transmission also contributes to the inhibition of itch, see later). The co-transmitter for glutamate was initially thought to be Grp, because gastrin releasing peptide receptor (Grpr) and Grpr-neurons are required for itch, and Grp-immunostaining was reported to be present in DRG (Sun & Chen, 2007; Sun et al, 2009; Zhao et al, 2013). Furthermore, the intrathecal administration of Grp (into the spinal cord) directly induces scratching behavior. Therefore, Grp was postulated to be a co-transmitter with glutamate in primary itch-responsive neurons (Sun & Chen, 2007). However, a number of reports have called into question the expression of Grp in DRG neurons (Fleming et al, 2012; Mishra et al, 2012; Mishra & Hoon, 2013) and it was suggested that Grp is instead expressed in spinal cord interneurons. The neuropeptide Nmb, is expressed at high levels in DRG neurons (Mishra et al, 2012; Wada et al, 1990). Nmb was suggested to act as a potential modulator of itch in one report (Sukhtankar & Ko, 2013), a finding that was not repeated in another study (Mishra et al, 2012). Therefore, a role of Nmb in itch-sensation is still controversial.

The search for the neurotransmitter used by DRG-neurons identified a new itch-specific neuropeptide, Natriuretic polypeptide b (Nppb;(Mishra & Hoon, 2013). Nppb, also called BNP, is a 32 amino-acid cyclic peptide that was discovered about 25 years ago (Seilhamer et al, 1989) (Figure 1). Nppb, as the name implies, is better known as a peptide that is released by the heart and controls blood sodium (and blood volume), activating its receptor in blood vessels and in the kidney. Nppb is part of a small gene family that encodes three distinct secreted peptides, Nppa, Nppb, and Nppc. Nppa and Nppb are both produced by heart muscle, are released into the blood, and activate the same receptor, Npr1 (Figure 1 and Table 1). The Nppc peptide activates a related receptor Npr2. Nppb was identified in DRG (Figure 2), by screening for genes that are preferentially expressed in cells that express or have developmentally expressed the capsaicin receptor, TRPV1(Mishra et al, 2011). This population of neurons was screened because, they have been shown to be required for itch-responses and because, the TRPV1-ion channel is essential for some types of itch-behavior (Imamachi et al, 2009). Analysis of the expression pattern of Nppb revealed that it is expressed in a subset of TRPV1-neurons. As expected for an itch neurotransmitter, Nppb is also co-expressed with the itch-receptors MrgprA3 and MrgprC11 (Liu et al, 2009; Liu et al, 2011), and is co-expressed with the critical downstream signaling molecule PLC β 3 (Han et al, 2006). The novel role for the natriuretic peptide Nppb was uncovered by analyzing the behavior of Nppb null mice. Animals lacking Nppb exhibit profoundly attenuated itch responses to all pruritogenic compounds tested, but reacted normally to other somatosensory stimuli. Nppb has also been reported to serve as a modulator of pain signals (Zhang et al, 2010) however, mice lacking Nppb display no nociceptive deficits contradicting this finding. Importantly intrathecal administration of Nppb recapitulates the same behavior as intradermal injection of pruritogens, namely it elicits scratching behavior in wild-type and Nppb knockout mice. Therefore Nppb has many characteristics of the bone-fide primary itch transmitter and, it was predicted that the receptor for Nppb, Npr1, should be expressed in interneurons located in the superficial layers of the dorsal horn. Indeed, consistent with Nppb acting as the transmitter for itch, Npr1-expressing neurons are present in the spinal cord (Figure 2). In addition, elimination of Npr1-neurons by intrathecal administration of Nppb-neurotoxin conjugate (Nppb-saporin) led to loss of itch reception while not effecting other somatosensory responses, including those produced by thermal and mechanical

stimuli. Together these data strongly support the proposal that Nppb and glutamate are the principle selective neurotransmitters used by pruritic sensory neurons.

2. Excitatory neurotransmission in the spinal cord

The dorsal horn of the spinal cord is well known to not only act as the conduit of pruritic, painful, and thermal input, but is also known to be a site where somatosensory afferent information is integrated. An indication of the complexity of the circuits in the spinal cord is provided by the demonstration of large number of transmitters that are present in this tissue (Su et al, 2014). Despite this apparent overwhelming complexity, significant headway has been made in unraveling the transmitters and cellular mechanisms involved in itch transmission.

The neurons which express the primary itch transmitter receptor, Npr1, are thought to be the first element in the itch neural circuit in the spinal cord (Mishra & Hoon, 2013). It was noticed that ablation of these neurons (using Nppb-saporin neurotoxin) did not eliminate Grp induced itch. This result is consistent with the transmitter Grp being downstream of Nppb. Furthermore, in mice lacking Nppb, intrathecal administration of Grp elicited normal itch-responses. What is more, antagonist blockade of Grpr, or elimination of Grpr-neurons inhibited equally Grp and Nppb elicited itch-behavior, concordant with a linear circuit for itch in the dorsal horn of the spinal cord (Figure 3). By assaying the expression of Grp, it was revealed that Grp is present at high levels in interneurons in the dorsal horn of the spinal cord (Figure 2) (Fleming et al, 2012; Li et al, 2006; Mishra & Hoon, 2013; Wang et al, 2013; Xu et al, 2013). To better define the neurons that express Grp, mice in which Npr1-cells are ablated were examined to see if the removal of these neurons leads to a concomitant reduction of Grp-expressing cells. As expected for the co-expression of Grp and Npr1, there was a dramatic reduction of Grp-expressing spinal cord neurons after Nppb-saporin treatment. Double labeling experiments further confirmed that Grp and Npr1 are co-expressed (Mishra & Hoon, 2013). The Grp/Npr1 class of neurons in addition to being stimulated by Nppb, is likely activated by glutamate (Koga et al, 2011). Therefore, there is strong evidence that Grp is downstream of Nppb and that Npr1-spinal cord interneurons are activated by the pre-synaptic release of Nppb and glutamate from DRG afferents (Figure 2). Nevertheless, the importance of Grp in the itch-pathway is undisputed; ablation of Grpr-neurons, elimination of the Grpr receptor itself, or administration of Grpr antagonist cause the selective loss of itch-responses (Sun & Chen, 2007; Sun et al, 2009). A recent pair of papers confirms the crucial roles of Grpr- and Grp-expressing interneurons in itch (Wang et al, 2013; Xu et al, 2013). The conditional deletion of the testicular orphan nuclear receptor 4, TR4, results in the loss of several types of dorsal horn neurons and also causes a dramatic reduction in responsiveness to itch-inducing agents. TR4 mutant mice have normal numbers of primary sensory neurons and projection neurons, and only show deficits in the number of excitatory dorsal horn neurons. The neurons lost in TR4 conditional knockouts include those expressing Grp and Grpr. Using a different genetic strategy, another study examined the consequence of loss of the T cell leukemia homeobox 3 gene, Tlx3, in dorsal horn neurons (Xu et al, 2013). Tlx3 conditional knockout mice lack a subset of spinal cord excitatory neurons in lamina I and II, including those which express Grp and Grpr. These mutant mice, like TR4 knockout animals, have lost responses to itch inducing agents. However, as both

these mutant mice have reduced responses to other somatosensory stimuli, assessment of the specificity of Grp and Grpr neurons and where they fit in itch-circuit models cannot be directly deduced from these studies.

Another excitatory neuropeptide that may be used by the spinal cord itch pathway is substance P (Stander et al, 2010; Trivedi & Bergasa, 2010). Although the gene that encodes substance P, Tac1, is not necessary for itch responses, several studies which used substance P antagonists reported that it can attenuate pruritic-behavior (Akiyama et al, 2014; Cuellar et al, 2003). In addition, the ablation of substance P receptor-expressing (NK-1) dorsal horn neurons with substance P-saporin also greatly reduced responses elicited by pruritic compounds (Carstens et al, 2010). Lastly, spinothalamic projection neurons which convey itch-signals from the spinal cord to the brain are immunopositive for the substance P receptor, NK-1 (Todd et al, 2000). The ablation of NK-1 expressing neurons causes a deficit in responses to painful stimuli as well as itch, which together with electrophysiological results has been interpreted to show convergence of sensory information in spinal cord projection neurons (Davidson et al, 2012; Davidson et al, 2009; Mantyh et al, 1997).

3. Inhibitory transmission in the spinal cord

Thermal, painful and mechanical stimuli can inhibit itch (Bromm et al, 1995; Yosipovitch et al, 2007). An important clue to the mechanism for the inhibition of itch by counter stimuli was obtained by examining the effect of silencing glutamate transmission from nociceptive neurons (Lagerstrom et al, 2010; Liu et al, 2010). By selectively abolishing glutamate neurotransmission (by eliminating VGlut2) in various classes of nociceptors, including those which express the voltage gated sodium channel Nav1.8, pruritogen induced itch was markedly increased while nociceptive responses were greatly reduced. As a note of caution, a comparable but not identical study in which other somatosensory specific conditional VGlut2 mice were examined, showed no change in itch-behavior in mutant animals (Scherrer et al, 2010). At present it is not possible to reconcile these differing results. Nevertheless, an explanation for the novel itch behavior described for Nav1.8 conditional VGlut2 mice was proposed in which these animals lose inhibition of pruritic input (Lagerstrom et al, 2010; Liu et al, 2010). It was suggested that normally tonic pruritic input is silenced by paired inhibitory nociceptive input. A model to account for this explanation posits that a class of inhibitory neurons is activated by nociceptive input; these cells in turn normally inhibit itch-signals (Figure 3). Indeed, this model is supported by mutant mice that lack a specific class of inhibitory neurons (Bhlh5I-cells), and display a similar spontaneous itch phenotype to that of conditional VGlut2 mutants (Ross et al, 2010). A search for the mechanism by which Bhlh5I-neurons inhibit itch revealed that they likely use the inhibitory transmitter, dynorphin, a κ -opioid receptor agonist (Table 1). Initially, it was shown that the κ -opioid receptor agonist nalfurafine inhibits scratching (Inan & Cowan, 2006; Kardon et al, 2014), suggesting that κ -opioid receptors are part of the inhibitory pathways for itch. Recently, it was shown that the endogenous κ -opioid receptor agonist, dynorphin, is expressed in Bhlh5I-neurons (Kardon et al, 2014). Grp induced itch is inhibited by nalfurafine suggesting that dynorphin probably inhibits either Grpr-neurons or cells further downstream in the itch pathway (Inan et al, 2011; Kardon et al, 2014). These results establishes that dynorphin is an important negative modulator of itch signals.

However, it must be noted that dynorphin knockout mice exhibit no itch phenotype, suggesting that there may be functional redundancy in the transmitters for inhibition or there is development plasticity which makes the role of dynorphin dispensable (Kardon et al, 2014). In addition to dynorphin, the neuropeptide somatostatin, (Sst), has been reported to induce itch-scratching responses (Kardon et al, 2014). A receptor for Sst, Sstr2 (Table 1), is found in many inhibitory neurons in the dorsal horn of the spinal cord (Polgar et al, 2013). Bhlhb5I-neurons co-express the Sstr2 receptor and can be hyperpolarized by application of Sst, suggesting that Sst may induce scratching by disinhibiting Bhlhb5-neurons. However, Sstr2 receptors are expressed in other classes of spinal cord interneurons, as well as in DRG-cells, and an alternate explanation of the inhibitory effects of Sst is that it triggers responses in these other classes of cells (Polgar et al, 2013; Shi et al, 2014; Takeda et al, 2007; Yasaka et al, 2010). In addition, the effects of Sst on nociception are well characterized, which given the interplay between itch and nociception input, could account for the induction of itch by Sst. Lastly, other inhibitors of itch are the neurotransmitters GABA and glycine (Akiyama et al, 2011). At present the exact source and cellular substrates for these transmitters is unknown. Cumulatively, we are beginning to see the outline of the inhibitory transmitters and interneurons that are responsible for the cross-talk between different sensory modalities which is crucial for the sentient perception of our environment.

4. Concluding remarks

In this review we have given our view of the itch circuit, which we believe is rather simple and is likely to be highly selective. However, we would like to stress that this model of itch is still a work in progress. As such, there may still be some surprises and there are also parts of the pathway that, while they are correct, lack detailed characterization. For instance, are the neuropeptides Nppb, Grp, dynorphin, and somatostatin neurotransmitters or neuromodulators? What is the precise anatomy of the neurons and how are they connected? Are some types of itch caused by perturbation of components in the itch circuit? In this rapidly moving field, these questions and more should be answered in the next few years and will build our knowledge of itch sensation and will ultimately produce a better understanding of the causes of chronic itch.

Much of the current work on itch has focused on the basic mechanisms by which signals are conveyed from peripheral pruritic-neurons to the spinal cord. Having gained these key insights, some of these breakthroughs are starting to be moved into translational studies. For instance, small molecular inhibitors of Grpr have been used to reduce itch in a mouse model (Zhao et al, 2013) and k-opioid agonist nalfurafine shows promise in the clinic at ameliorating uremic pruritus (Kumagai et al, 2012; Wikstrom et al, 2005). Similarly, antagonists of Npr1 and Sstr2 receptors may be tractable alternative strategies for treatment. However, without exception, all the transmitters in the itch circuit perform “double-duty” as components in non-itch processes in either other areas of the brain, or in other parts of the body. Therefore, drugs which interfere with the receptors for these transmitters might produce unwanted side-effects and while not necessarily precluding their usefulness, these off-target effects may curtail their utility in the clinic.

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Abbreviations

Nppb	Natriuretic polypeptide b
Nmb	Neuromedin b
Grp	Gastrin releasing peptide
Npr1	Natriuretic peptide receptor 1
Npr2	Natriuretic peptide receptor 2
Grpr	Gastrin releasing peptide receptor
Sst	Somatostatin
DRG	Dorsal root ganglion
TRPV1	Transient Receptor Potential sub-family Vanniloid member 1
VGlut2	Vesicular glutamate transporter 2
TR4	Testicular orphan nuclear receptor 4
Tlx3	T cell leukemia homeobox 3
NK1	Neurokinin 1
Sstr2	Somatostatin receptor 2
Bhlh5I	Basic helix loop helix transcription factor 5 inhibitory neurons
PLC	Phospholipase C

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Natriuretic peptide	Amino-acid sequence
mNppb	NSKVTHISSCFCHKIDRISSVSRLCNALKLL
hNppb	SKMVQGSICFCRKMDRISSSSLCCKVLRHH
rNppb	NSKMAHSSSCFCNKIDRICAVSRLCDGLRLF
mNppa	GRSLRRSSCFGGRIIRICAQSSLCNSFRYRR
hNppa	APSLRRSSCFGGRMIRICAQSSLCNSFRY
rNppa	GRSLRRSSCFGGRIIRICAQSSLCNSFRYRR

Figure 1.
Structure of Natriuretic peptides.

A comparison of the primary structure (table) of mouse, human, and rat natriuretic polypeptides reveals that Nppa displays greater sequence conservation than Nppb. Despite these differences, both Nppa and Nppb activate the same receptor; Npr1. Amino acids are color coded to reflect their characteristics. The structure of the natriuretic peptides is schematized (to the right) to highlight the core conserved ring that forms the key structural element of these transmitters. Only amino-acid residues that have high sequence conservation between species and between Nppa and Nppb are colored. The cysteine residues and their critical disulfide bond are marked in yellow. The diagram also illustrates that the N-terminal and C-terminal loops exhibit low sequence identity and are not thought to be important determinants in receptor binding.

Figure 2.

Expression pattern of itch-specific neurotransmitters and their receptors.

In situ hybridization shows the neurons which express the transmitters Nppb and Grp and their respective receptors, Npr1 and Grpr. Nppb is expressed in about 6% of DRG neurons that have small diameters. The receptor for Nppb, Npr1, is expressed in dorsal horn neurons. Likewise Grp and Grpr are also expressed in the superficial dorsal lamina of the spinal cord.

Figure 3.
Schematic of the Itch-circuit.

Schematic representation of the distribution and function of itch-selective transmitters and their neuronal targets illustrates the simplicity of the itch-circuit.

Excitatory pathway (blue colored cells): 1) Glutamatergic and Nppb excitatory input from pruritic neurons activates Npr1-expressing spinal cord neurons. 2) Npr-1 expressing neurons release Grp which in turn activate tertiary pruriceptive neurons that express the Grpr receptor. 3) The transmitter used by Grpr-expressing neurons has not yet been identified, however, substance P (Tac1) is believed to play a role in activation of the last step in the spinal cord itch circuit; the stimulation of spinothalamic projection interneurons.

Inhibitory pathway (green colored neurons): 1) Glutamatergic afferents of nociceptors (red colored neurons) stimulate Bhlhb5-inhibitory neurons. 2) Bhlhb5-expressing neurons release dynorphin (Pdyn) which activates k-opioid receptors in post-synaptic membranes of unknown pruritic pathway neurons. 3) Somatostatin (Sst) from dorsal horn- or DRG-neurons activates and in turn inhibits neurons which express the Sstr2 receptor. Since Sstr2 leads to reduced cell-activity, Sst mediated activation of these neurons causes disinhibition and therefore potentiates itch-behavior. The inhibitory neurotransmitters GABA and glycine are also involved in reducing itch-responses. The segments of the pathway that are unknown or controversial are marked in red.

Table 1

Table of the major itch-neurotransmitters and their receptors: The receptors for the four major itch neurotransmitters are listed together with a partial list of their known agonists and antagonist, as well as their coupling to cellular signaling cascade. Notably, Npr1 and Grpr receptors stimulate excitatory signaling processes which cause elevation of intracellular concentrations of cGMP or PLC metabolites respectively. In contrast, the Sstr2 and Opkr1 receptors activate downstream components which cause a lowering of intracellular cAMP concentration and produce a inhibition of neuronal firing. Abbreviations: Nor BNI–norbinaltorphimine and 5'GNTI-5'-guanidinonaltrindole.

Receptor	Gene name	Agonist	Antagonist	Transducer
Natriuretic peptide receptor A	Npr1	Nppa (Atlas et al, 1985) Nppb (Tse et al, 2001)	Anantin ^(Wyss et al, 1991)	Guanylyl cyclase (Garbers & Lowe, 1994)
Gastrin releasing peptide receptor	Bb2	GRP (Jensen et al, 2008)	BIM 189 (Coy et al, 1990)	Gq/G ₁₁ family (Hellmich et al, 1997)
Somatostatin receptor 2	Sstr2	Sst (Kardon et al, 2014) Octreotide (Raynor et al, 1993)	CYN-154806 (Bass et al, 1996)	G _i /G _o family (Hou et al, 1994)
Kappa opioid receptor	Opkr1	Dynorphin (Kardon et al, 2014) Nalfurafine, U-50, 488 (Morgan & Christie, 2011)	Nor BNI, 5'GNTI (Morgan & Christie, 2011)	G _i /G _o family (Dhawan et al, 1996)