Published in final edited form as:
Mol Immunol. 2015 January ; 63(1): 38–44. doi:10.1016/j.molimm.2014.03.001.

Mast cells: versatile gatekeepers of pain

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Abstract

Mast cells are important first responders in protective pain responses that provoke withdrawal from intense, noxious environmental stimuli, in part because of their sentinel location in tissue-environment interfaces. In chronic pain disorders, the proximity of mast cells to nerves potentiates critical molecular cross-talk between these two cell types that results in their synergistic contribution to the initiation and propagation of long-term changes in pain responses via intricate signal networks of neurotransmitters, cytokines and adhesion molecules. Both in rodent models of inflammatory pain and chronic pain disorders, as well as in increasing evidence from the clinic, it is abundantly clear that understanding the mast cell-mediated mechanisms underlying protective and maladaptive pain cascades will lead to improved understanding of mast cell biology as well as the development of novel, targeted therapies for the treatment and management of debilitating pain conditions.

Keywords

mast cells; pain; cytokines; neurons

1. Introduction

Mast cells are important cellular regulators of physiological and pathological pain pathways. Pain can be either protective or maladaptive. Protective pain can be further categorized as nociceptive pain that signals for the removal of an intense stimulus, or inflammatory pain associated with tissue damage that promotes immune cell infiltration and hypersensitivity until tissue repair is achieved[1]. Pathological, maladaptive pain is chronic in nature, and is associated with prolonged disease states [1]. The interaction between the nervous system and the immune system plays a pivotal role in pain processing[2]. Mast cells are particularly important in this regard as they are frequently found in close proximity to nociceptive neurons[3]and therefore can participate in juxtacrine signaling in neuro-immune synapses [3]. Mast cells induce nociceptor activation through the release of

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chemical mediators during degranulation and can be activated by mediators released from nociceptors upon injury[2, 3].

2. Mast cell abundance and activity are reported in various clinical pain disorders

Several associations have been reported between mast cell activation and abundance and clinical pain disorders (Box 1). Migraine attacks were reported to occur with higher frequency in asthma and allergy patients [4] and plasma histamine levels were found to be elevated in a 20-patient sample of migraine patients vs. controls both during headache attacks and symptom-free periods [5], likely suggesting a role for mast cell activation in migraine onset. Bladder mast cell activation was confirmed by electron microscopy in 26 of 52 interstitial cystitis patients [6] while mast cell-derived tryptase levels were increased in expressed prostatic secretions in men (6–7 patients vs. 5 controls) with chronic pelvic pain syndrome [7]. Tryptase levels were also increased in the skin of the affected extremities of patients with complex regional pain syndrome (CRPS); 43 patients undergoing elective hand surgery followed by cast immobilization had upregulated mast cell tryptase levels in the skin of hands ipsi-lateral to surgery [8]. Patients with CRPS also showed dysregulated substance P release [9]. Substance P (SP) activates mast cells through the neurokinin-1 (NK-1) receptor [3]. Mast cells themselves can release SP; immuno-co-localization of SP has been reported in cutaneous mast cell granules of 18 atopic dermatitis patients compared to 10 controls [10].

Box 1

Clinical pain disorders associated with mast cells

Migraine [4,5]

Pelvic and bladder pain [6,7]

Atopic dermatitis [10]

Inflammatory bowel pain [19, 20]

Fibromyalgia [15]

Vulvodynia [11–14]

Complex regional pain syndrome [8, 9]

Self-injurious behavior associated pain [16]

Vulvodynia, a chronic vulvar pain disorder, has been epidemiologically associated with a self-reported history of allergies [11]. An increased number of mast cells overall, and more degranulated mast cells, as well as greater epithelial innervation were detected in vestibular biopsies of 40 women with vulvodynia [12] compared to biopsies from 7 controls. A follow-up study showed that tissue samples from 7 vulvodynia patients showed higher levels of heparanase activity compared to 7 controls [13]. These studies were corroborated by independent findings of increased mast cell numbers and innervation in tender vs. non-

tender vestibular sites in 10 patients with primary, provoked vulvodynia [14]. Significant increases in numbers of skin mast cells at tender sites have been reported in a cohort of 63 patients suffering from fibromyalgia, a syndrome characterized by skeletal muscle pain and headache along with fatigue and sleep disorders, vs. an age-matched cohort of 49 controls [15]. Similarly, skin biopsies from 16 non-verbal, cognitively disabled adults with chronic self-injurious behavior showed significantly more degranulated mast cells and innervation at body-matched non-injury sites when compared to 9 disability-matched controls without self-injurious behavior [16]. In addition, individuals with self-injurious behavior displayed increased sensitivity to thermal and mechanical stimuli applied 15 cm below the skin biopsy site, compared to disability-matched controls without self-injurious behavior [16]. Responses to painful stimuli were assessed via the Facial Action Coding System; blinded coders identified the absence or presence, intensity and temporal features of facial muscle movements [16].

There is emerging evidence that gastro-intestinal inflammatory diseases, frequently associated with abdominal pain and distress, are associated with histories of atopy [17] and accompanied by mast cell infiltration [18]. Barbara and colleagues reviewed multiple studies (with patient cohorts ranging from 4 to 77) that showed increased intestinal mucosal mast cell infiltration into the ileum, colon and rectum in patients with inflammatory bowel syndrome; these mast cells were observed to be in close contact with neurons, and their abundance correlated with the severity of perceived abdominal pain symptoms [19]. Gastrointestinal biopsies from a cohort of 48 children with inflammatory and non-inflammatory chronic abdominal pain found an inverse relationship between mast cell counts and levels of IL-6 and SP; mast cell numbers were elevated in the colon mucosa of patients with non-inflammatory pain [20]. In a cohort of 69 women undergoing laparoscopic excision of endometriosis for pain vs. 37 controls, significantly higher numbers of active and degranulating mast cells were reported in deep infiltrating lesions, and to a lesser extent in peritoneal and ovarian lesions, with mast cells localized <25μm from nerves [21], suggesting that direct nerve-mast cell interactions could contribute to the painful pathology of this condition.

Taken together, multiple lines of evidence indicate that mast cells may be important regulators of pain pathologies, and these findings have inspired investigators to build mast cell-focused pre-clinical models of pain disorders to help elucidate the relevant underlying mechanisms.

3. Mast cell activation provokes experimental thermal and mechanical hyperalgesia in rodents

Changes in rodent behavioral responses to acute, noxious environmental stimuli are measured in classic assays of hypersensitivity – hyperalgesia (response to a painful stimulus) or allodynia (painful response to a previously tolerated stimulus) [22]. While these are imperfect assessments of psychologically and physiologically complex human pain states, they provide tractable models for interrogating cellular and molecular mechanisms underlying altered sensitivities to temperature and pressure stimuli [22, 23]. These

approaches can be used to elucidate the mechanistic contributions of mast cells to the pathophysiology of acute and chronic pain conditions (Box 2).

Box 2

Mast cell-focused rodent models of pain

- Chemically induced heat and pressure sensitivity [66, 67]
- Passive cutaneous anaphylaxis pain [30, 32]
- Compound 48/80-induced migraine [38–40]
- Experimental cystitis and prostatitis [7, 42–44]
- Venom-induced hyperalgesia [27, 51, 52]
- Oxazolone-induced vulvar pain [54]
- Post-operative pain [46, 47, 48]
- Neuropathic pain and Complex Regional Pain [61–64]
- Sickle cell disease-associated pain [65]

We have recently shown that genetically mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice have significantly reduced thermal and mechanical plantar hyperalgesia after intra-plantar injection with mast cell secretagogue compound 48/80 (c48/80) compared to wild-type (WT) mice; these responses were restored following tissue-specific reconstitution of the hind paw with bone marrow cultured mast cells [24]. Deficiencies in c-kit cause defects other than reduced mast cell numbers that may have differential effects on various pathophysiological outcomes [25]; it has been reported that C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice are hypo-responsive to vertically applied punctate heat stimuli [26]. However, we found that baseline responses to both a thermal stimulus applied (via a 50°C hotplate) across all four paws, and vertically applied punctate mechanical pressure using an electronic pressure meter [24, 27], were indistinguishable between C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$, mast cell-reconstituted and WT littermates. These mast cell-dependent acute nociceptive responses were dependent on histamine signaling and neutrophil influx into the inflamed tissue [24]. Compound 48/80-induced mast cell degranulation provokes similar nociceptive pain responses in ND4 Swiss mice [24]; interestingly, when these mice were systemically pre-treated with TNF-α-neutralizing antibodies, these acute early nociceptive responses were rapidly blocked even though infiltrating neutrophils were present [28]. It is possible that TNF-α released from mast cell granules have rapid effect on nociceptors that contribute to immediate protective nociceptive responses. These findings support an earlier observation that injection of c48/80 into the forearms of young male volunteers caused significant changes in pain sensitivity in the skin surrounding the sites of administration [29].

In a model of passive cutaneous anaphylaxis(PCA) in the rat hind paw, Lavich and colleagues[30] demonstrated a state of transient increased sensitivity to vertically applied punctate heat (lasting 1–3 hours)following plantar anti-dinitrophenyl (DNP)-IgE sensitization and DNP challenge. While the authors did not characterize mast cell

contributions *per se*, mast cell degranulation is the primary orchestrator of downstream effects of IgE-DNP crosslinking [31]. These thermal hyperalgesic responses were regulated by the synergistic interaction of histamine, br adykinin, and serotonin – all likely products of mast cell degranulation[30]. Our unpublished data indicate that pronounced thermal *and* mechanical hind paw hyperalgesia caused by PCA reactions in the footpad last up to 6 hours in mice and are accompanied by moderate to extensive mast cell degranulation (manuscript in preparation). In a subsequent study, Lavich and colleagues, without specifically assessing mast cell activity, showed that rats challenged with ovalbumin (OVA) after sensitization with OVA and Al_3OH (alum) showed thermal pain responses that lasted for 6 hours, and were mediated by infiltrating neutrophils [32]. Earlier, Piovezan and colleagues also demonstrated a reduction of OVA/alum-induced hyperalgesia by prior chemical depletion of mast cells (using repeated c48/80 administrations) in the hind paws of sensitized mice[33], demonstrating that mast cell degranulation can contribute to pain resulting from active sensitization and antigen challenge. Mast cell depletion by c48/80 pre-treatment as well as serotonin antagonist administration separately reduced formalin-induced secondary allodynia and hyperalgesia in rats implicating mast cell-derived serotonin signaling in the maintenance of pain behaviors [34]. Pre-administration of histamine receptor antagonists as well as sodium cromoglycate (SCG) – a compound widely used as a mast cell granule stabilizer – also reduced later phases of formalin pain [35]. It is important to remember that SCG can have direct effects on nerves [36] and can also have differential effects on mast cell degranulation depending on the experimental system [37]. Therefore, all experimental evidence that uses SCG as a mast cell-tropic reagent should be interpreted with caution unless accompanied by suitable controls or follow-up experiments conducted in mast cell-deficient conditions.

4. Mast cells in pre-clinical models of inflammatory and chronic pain disorders

4.1. Migraine

Levy and colleagues demonstrated electrophysiological activation of meningeal nociceptors following dural mast cell degranulation in rats, suggesting dural mast cell activation as an underlying mechanism of migraine pathology [38]. Systemic c48/80 administration was used to activate dural mast cells, while pERK and c-fos activation in the dura were used to measure prolonged activation of the trigeminal pain pathway [38]. Compound 48/80-provoked nociceptive signaling was blocked by pre-administration of SCG. Further studies from these investigators showed tactile pain at different locations in the body indicating the activation of central sensitization mechanisms; the authors demonstrated this by showing c-fos expression at different levels in the spinal cord following systemic mast cell activation [39] and identified relevant roles for mediators including TNF-α, IL-1β and IL-6 that can be released by degranulating mast cells [40]. Different N-truncated fragments of known migraine inducer pituitary adenylate cyclase activating peptide-38 (PACAP-38) have been shown to cause differential levels of peritoneal and dural mast cell degranulation in rats, thus pointing to mast cell degranulation as a critical component of PACAP-38 induced migraine headaches [41].

4.2. Interstitial cystitis and pelvic pain

Mast cells contribute to mechanical cystitis pain via histamine receptor 1 and 2 signaling in a murine model of pseudorabies virus (PRV)-induced pelvic pain [42]. In this study, cystitis pain was abrogated in C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice and restored with whole bone marrow transplantation. However, these experiments did not specifically reconstitute mast cells in the bladder and therefore did not isolate mast cells as the relevant cellular players; it is possible that repair of other c-Kit-related defects may have played unspecified roles in the restoration of pain [42]. NK-1 and histamine receptor 2 antagonists mitigated bladder-associated pelvic pain in this model, suggesting that blockade of potentially mast cell-mediated regulatory mechanisms *i.e.* histamine and SP signal pathways, may have therapeutic potential in the treatment of cystitis-related pain [43]. Chemotactic cytokines, chemokine ligands 2 and 3 (CCL2 and CCL3), have been identified as important mediators in pelvic pain associated with a murine model of experimental prostatitis [44]. CCL2 can also induce histamine release that is subsequently associated with the development of cystitis-related pain in rats [45].

4.3. Post-operative pain

Oliveira and colleagues observed massive plantar degranulation following incision injury in mice accompanied by significant mechanical sensitivity; mast cell depletion via c48/80 pre-treatment, pre-treatment with mast cell stabilizer SCG, and combined blockade of histamine and serotonin signal pathways each separately reversed post-operative pain in these mice [46]. In a follow-up study, the release of mast cell tryptase following degranulation, and subsequent activation of proteinase-activated-receptor 2 (PAR-2) were shown to contribute to the changes in pain sensitivity [47]. Yasuda and colleagues corroborated these findings and observed that post-incision allodynia and guarding pain, but not heat sensitivity, were reduced by pre-treatment with SCG [48].

4.4. Venom-induced hyperalgesia

Mast cells are key neutralizers of various insect, scorpion and reptile venoms as well as structurally related mammalian peptides [49, 50, 51]. *Bothrops jararaca* venom administration caused marked mast cell degranulation and acute mechanical sensitivity in the hind paws of mice [27]; Bonavita and colleagues demonstrated that mast cell stabilizer SCG inhibited *Bothrops jararaca* venom-induced release of histamine and leukotriene C4 *in vitro* and abrogated venom-induced hyperalgesia *in vivo* in rats [51]. *Buthus martensi* Karch scorpion venom induced mast cell degranulation and nociceptive behaviors in rats; these behaviors were reduced following mast cell depletion via chronic administration of c48/80 [52].

4.5. Vulvodynia

We adapted an established model of mast cell-dependent contact hypersensitivity to topically applied hapten oxazolone [53], and found increased vulvar tactile sensitivity in oxazolone (Ox)-sensitized female mice after single and repeated labiar skin challenges with Ox [54]. Female ND4 Swiss mice were sensitized with topical oxazolone on their flanks and challenged 1–3 times on the labia on day 5 or day 5–7, respectively. After a single

challenge, heightened mechanical sensitivity of the ano-genital ridge lasted 24 hours and accompanied hyperinnervation, neutrophil influx, and increased expression of inflammatory cytokine genes in the labiar tissue [54]. Three daily oxazolone challenges produced vulvar mechanical hyperalgesic responses and increases in nerve density that were detectable up to 5 days post-challenge even after overt inflammation (neutrophil influx and upregulation of inflammatory cytokine transcripts) had resolved. Both the sensitization phase of Ox-CHS [53, 55] and changes in cutaneous nerves during the elicitation phase [56] have been previously shown to be mast cell-dependent.

The persistent hyperalgesia and sustained hyper-innervation seen in our experimental animals mirror the clinical hallmarks of provoked vulvodynia [12, 13, 14] and provide a tractable model to experimentally dissect the epidemiological evidence that correlates a history of allergic reactions, most notably hives and exaggerated reactions to insect bites with an increased risk of developing vulvodynia [11] through potentially mast cell-dependent mechanisms. We are currently studying the effects of long-term re-exposures to allergen to assess the effects of mast cell accumulation in hyper-innervated tissues to the overall increase in peripheral and central pain cascades that contribute to chronic vulvar pain. In the clinic, contact dermatitis and other skin disorders (modeled in rodents with contact hypersensitivity reactions) include both itch and pain sensations [57]. Until recently, itch and pain have been difficult to distinguish on a behavioral basis in rodents. In our studies, we defined pain (nociceptive) responses as licking or jumping following mechanical stimulation [54]. Licking of the affected region or wiping it with forepaws has been shown to correlate with pain sensation, while biting correlated more with itch [58, 59]. Our unpublished data suggest that Mas-related G-protein coupled receptor A3 (MrgprA3)$^{+}$ expression is not significantly enhanced in painful sites multiply challenged with oxazolone in mice exhibiting vulvar tactile sensitivity. MrgprA3 was recently shown to be a marker of itch-sensing (pruritoceptive) neurons, as depletion of Mrgpr3A^{+} neurons abrogated responses to well-characterized pruritogenic agents, but not heat, pressure, or cold stimuli (that normally elicit pain) [60].

4.6. Neuropathic pain

Beyond individual disease models, investigators are also using rodents to model complex and co-morbid pain conditions such as neuropathic pain, complex regional pain syndrome (CRPS) and sickle cell disease. Pre-treatment with SCG inhibited mast cell degranulation, neutrophil and macrophage infiltration as well as thermal and mechanical hyperalgesia caused by the ligation of the sciatic nerve in rats; furthermore, neuropathic hyperalgesia was reduced after treatment with histamine 1 and 2 receptor antagonists [61]. Taiwo and colleagues observed that following the ligation of the 5th lumbar spinal nerve, neuropathic hyperalgesia and thalamic mast cell abundance in the side contra-lateral to ligation increased in female but not in male mice suggesting a role for mast cell-dependent nociceptive signaling in the CNS [62]. In a model of paclitaxel-induced neuropathy in mice, repeated chemotherapy administration caused an increase in mast cell tryptase activity in the spinal cord and increased thermal and mechanical sensitivity that was abrogated by blockade of signal pathways downstream of PAR2, or by treatment with antagonists of transient vanilloid receptor potential (TRP)V1, TRPV4 or TRP ankyrin (TRPA)1 [63].

Li and colleagues have developed a rat model of Type 1 CRPS in which tibia fracture induced in the right hind limb caused post-fracture mechanical nociception accompanied by SP-induced mast cell accumulation, activation and degranulation. These effects were inhibited by NK-1 receptor antagonist treatment suggesting a role for peptidergic neuron-mast cell signaling in this chronic pain syndrome [64]. Mast cells were recently found to be important contributors to the development of cutaneous and deep hyperalgesia as well as hypoxia-reperfusion-induced pain in transgenic sickle mice (expressing >99% human sickle hemoglobin) primarily through a tryptase-PAR2 pathway of amplified neuropeptide release [65].

5. Mast cell-neuron synapse in tissue contribute to pain responses

Taken together, the aforementioned clinical and pre-clinical evidence point to mast cell-regulated pathways as important mediators of pain pathologies. Elucidation of the underlying molecular mechanisms from the analysis of nerve-mast cell interactions in steady-state and painful tissues (summarized in Figure 1) are essential next steps in the identification of biomarkers and therapeutic targets that can be used to classify, manage and treat acute and chronic pain conditions.

Mast cells release a variety of mediators such as histamine, serotonin, IL-1β, TNF-α, and IL-6, all of which have the ability to independently induce [66, 67] or mediate chemical- [67], infection- [42, 43], or allergen-evoked hyperalgesia [30, 32] either via direct effects on nociceptors or by stimulating the production of final mediators such as leukotrienes and prostanoids [66]. Furthermore, mast cells residing in close proximity to unmyelinated nerve fibers, such as the nociceptive C-fibers, can undergo ultrastructural alterations that allow differential or selective “piecemeal degranulation” [3]. Co-cultures of bone marrow cultured mast cells and superior cervical ganglia reveal that association with neurites increases IgE/antigen (Ag) crosslinking-induced calcium signaling in mast cells associated with increased surface expression of FcεR1α [68]. Cutaneous nerve depletion *in vivo* abrogates PCA-induced tissue edema and endothelial permeability [69]. The observed reduction in PCA responses is not due to altered numbers of mast cells, but rather impaired mast cell activation by IgE/Ag cross-linking in the absence of cutaneous nerves [69]. Taken together, these studies suggest that allergy-induced mast cell activation depends on neuronal cues. This is particularly important for understanding pain conditions where anatomical mast cell-nerve associations have been documented in addition to atopic history, such as inflammatory bowel syndrome [17] and vulvodynia [12, 13, 14].

Adhesion molecules such as N-cadherin and cell adhesion molecule-1 (CADM-1) facilitate mast cell-nerve junctions [70, 71, 72]. N-cadherin mediated-mast cell-nerve interaction is regulated by matrix metallo-proteinase MMP24; Mmp-24 deficiency abrogates acute thermal hyperalgesia by altering neuron-mast cell synapses [73]. Mast cell mediators NGF and TNF-α influence neuronal growth, and both can lower the threshold of nociceptor firing through binding to TRK1-transforming tyrosine kinase protein (trkA) and TNF receptor, respectively [56,74, 75, 76]. Another important signal at the mast cell-nerve synapse is substance P. Released by both neurons and mast cells, substance Pleads to production of histamine, prostaglandins and leukotrienes, as well as TNF-α and IL-6 by mast cells [10, 77,

78, 79]. Substance P receptor NK-1 is not constitutively expressed on all mast cell subtypes, but its expression can be induced by IgE/Ag crosslinking, suggesting a mechanistic association between allergies and pain [80].

As mentioned above, antagonism of substance P receptor NK-1 abrogates cystitis pain in mice [43]. Interestingly, substance P-NK-1 binding primes mast cells to degranulate upon repeated application of lower doses of substance P [78]. In addition, tryptase released by mast cells can cause hyperalgesia in rats upon binding to PAR2 on nociceptors [81] as shown in several rodent models of pain discussed above [63, 65]. PAR2-mediated hyperalgesia depends on TRPV4 in a model of colorectal distension [82]. Moreover, tryptase-induced PAR2 activation triggers TRPV1 and TRPV4 sensitization via phospholipase C (PLC), phosphokinase A (PKA) and phosphokinase Cε(PKCε) [81, 82]. PAR2 activation can induce CGRP release [83], which in turn stimulates histamine release from dural mast cells [84]. This pathway has been proposed as the underlying mechanism of migraine [79], further supported by the efficacy of CGRP antagonists in migraine treatment [85].

6. Conclusion

Mast cells reside in sentinel locations in the tissue and release a versatile repertoire of mediators [86] including those that interact structurally and functionally with pain-sensing nociceptors [3]. There is ample evidence that mast cell contributions to pain pathologies is an active area of biomedical investigation in discovery research as well as in the clinic.

Currently, much of the evidence points to strong associations rather than specific mechanisms of action that implicate mast cells as necessary for the induction of certain kinds of pain. Many excellent mast cell-focused models of acute, inflammatory, chronic and neuropathic pain have been established. Now these approaches must be validated in the context of mast cell-deficiency and reconstitution. Several newly described conditional mast cell knockout strains of mice [25] are potentially useful tools to screen for truly mast cell-dependent pain disorders where mast cell-targeted therapies may be most effective and beneficial.

One area where an understanding of specific mast cell contributions to pain may substantially transform our current understanding and therapeutic approaches is that of pain conditions (such as fibromyalgia, subsets of vulvodynia or inflammatory bowel pain) that present without overt inflammation. Central sensitization and CNS amplification and maintenance of pain states can be seen as distal and disconnected from inflammatory processes [87]. However, mast cell associations with such conditions have been reported in multiple clinical contexts [12–14, 19–20]. The findings from pre-clinical models that systemic mast cell activation can cause pain at different locations in the body [39], thalamic mast cell abundance can change in response to nerve ligation [62] and increasing evidence that mast cell-glial crosstalk in the CNS mediate neurodegenerative processes [88] all point to the possibility that these versatile immune effectors may well be important players in maintenance and transmission of central pain as has been recently reviewed [89].

Systematic elucidation and analyses of mast cell contributions to central and peripheral pain mechanisms will deepen our understanding of mast cell biology as well as aid the design of novel, rational therapies for the treatment and management of pain.

Acknowledgments

Alyssa Ashbaugh contributed to an earlier draft of this review. We also thank Jyothi Dhanwada, Emily Ewan, Modestas Filipavicius, Eyerusalem Lemma, Antsa Randriamihaja, and Kidist Zewdie for their assistance. DC is supported by Macalester College, the National Institutes of Health (R15 NS067536-01A), and the National Vulvodynia Association.

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Highlights

- Mast cells are increasingly associated with pathophysiology of pain disorders
- Mast cell-focused pre-clinical rodent models of pain can reveal relevant mechanisms
- Mast cell-neuron synapses in the tissue modulate nociceptive signal cascades
- Mast cell contributions to central and peripheral pain will inform new therapeutics

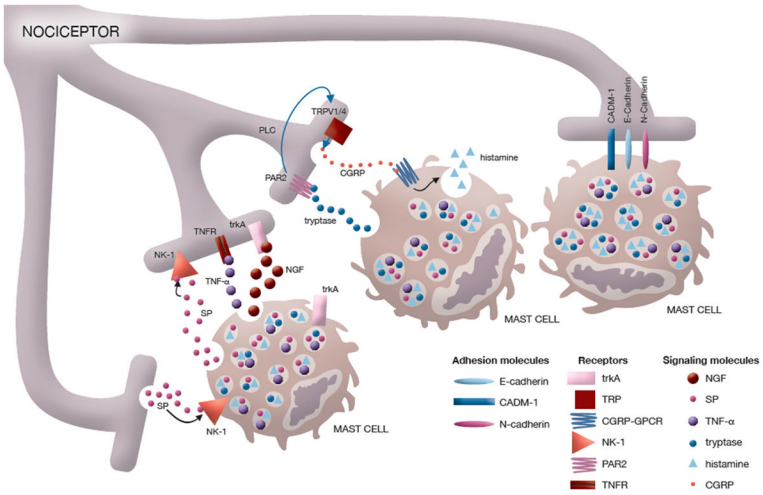

Figure 1. Mast cell-nerve proximity in the tissue facilitates neuro-immune cross-talk relevant to modulation of pain

Mast cell-nerve interactions are facilitated by adhesion molecules E-Cadherin, CADM-1, and N-Cadherin [62, 63]. Both mast cells and neurons have the potential to secrete nerve growth factor (NGF) and substance P (SP) that bind trkA, and NK-1 receptors, respectively [3]. NGF and SP participate in nociceptive signaling. NGF and tumor necrosis factor-α (TNF- α) secreted by mast cells induce neuronal growth, and reduce subsequent neuronal firing threshold through binding with trkA and TNFR, respectively [55, 65, 66, 67]. Tryptase released by mast cells binds to proteinase-activated receptor 2 (PAR2) on neurons, and initiates a cascade involving TRPV1/4 activation via PLC and release of CGRP [72, 73]. CGRP in turn binds to a G-protein coupled receptor (CGRP-GPCR) on mast cells, and promotes histamine release [74, 75]. [*Artwork: John Koenig*]