Biochemical and Biophysical Research Communications 425 (2012) 237–243

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Mast cell degranulation mediates compound 48/80-induced hyperalgesia in mice

Devavani Chatterjea *, Abigail Wetzel, Madison Mack, Camilla Engblom, Juliann Allen, Carolina Mora-Solano, Luisa Paredes, Evelyn Balsells, Tijana Martinov

*Department of Biology, Macalester College, USA*

ARTICLE INFO

*Article history:*
Received 13 July 2012
Available online 22 July 2012

*Keywords:*
Mast cell
Compound 48/80
Histamine
Hyperalgesia
Neutrophil

ABSTRACT

Mast cells mediate allergies, hypersensitivities, host defense, and venom neutralization. An area of recent interest is the contribution of mast cells to inflammatory pain. Here we found that specific, local activation of mast cells produced plantar hyperalgesia in mice. Basic secretagogue compound 48/80 induced plantar mast cell degranulation accompanied by thermal hyperalgesia, tissue edema, and neutrophil influx in the hindpaws of ND4 Swiss mice. Blocking mast cell degranulation, neutrophil extravasation, and histamine signaling abrogated these responses. Compound 48/80 also produced edema, pain, and neutrophil influx in WT C57BL/6 but not in genetically mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice. These responses were restored following plantar reconstitution with bone marrow-derived cultured mast cells.

© 2012 Elsevier Inc. All rights reserved.

## 1. Introduction

Immune cells contribute to acute and chronic pain in diverse and nuanced ways [1]. Mast cells are versatile effectors [2] whose roles have been studied in rodent models of migraine [3], interstitial cystitis [4], and postoperative pain [5], as well as in clinical studies of irritable bowel syndrome [6], endometriosis [7], and thermal capsaicin pain [8]. Peripherally located at tissue-environment interfaces, mast cells respond to a wide array of stimuli by promptly releasing a spectrum of both preformed and newly synthesized mediators [1]. Treatments that modulate the activities of mast cell mediators can alter inflammation-associated thermal and mechanical pain responses induced by zymosan and acetic acid [9], formalin [10], and venom [11].

Systemic mast cell degranulation in rats produced c-fos activation in medullary and dorsal horn neurons in a rat model of mechanical sensitivity [12]. Mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ (Wsh/Wsh) mice exhibited decreased pseudorabies virus-induced pelvic pain compared to wild-type (WT) C57BL/6 mice, and injections of WT bone marrow restored the histamine-mediated pain responses [4]. However, there has been little evidence in mice that localized mast cell degranulation can directly cause measurable pain.

We found that localized plantar mast cell degranulation produced thermal hyperalgesia, edema, and neutrophil influx in ND4 Swiss mice. Mast cell granule stabilization, inhibition of neutrophil influx, and histamine receptor antagonism inhibited nociceptive behaviors. Compound 48/80-mediated thermal and mechanical hyperalgesia observed in WT C57BL/6 mice were abrogated in mast cell-deficient Wsh/Wsh mice and restored in Wsh/Wsh mice with hindpaws locally reconstituted with cultured mast cells.

## 2. Materials and methods

### 2.1. Animals

Three–six months old male ND4 Swiss and C57BL/6 mice (Harlan Laboratories, Indianapolis, IN), mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ (Wsh/Wsh) mice and mast cell-reconstituted C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ (Wsh/Wsh:WT) (gift of Dr. Stephen Galli, Stanford University) were housed in Macalester College's animal facility with a 12-h light/dark cycle with food and water *ad libitum*. Bone marrow-derived cultured mast cells ($2 \times 10^6$/hindpaw) were transplanted into Wsh/Wsh mice [13]; age-matched controls received saline [14]. Reconstituted mice were used >12 weeks post-transplant. Macalester College's Institutional Animal Care and Use Committee approved all experimental procedures.

### 2.2. Drug administration

All drugs (Sigma–Aldrich, St. Louis, MO) were administered using 0.9% saline vehicle. Mice received bilateral intraplantar

*Abbreviations:* WT, wild-type; c48/80, compound 48/80; MPO, myeloperoxidase; H1R, histamine receptor 1; H3/4R, histamine receptor 3/4; SCG, sodium cromoglycate.

* Corresponding author. Address: Department of Biology, Macalester College, 1600 Grand Avenue, St. Paul, MN 55105, USA. Fax: +1 6516966443.
*E-mail address:* chatterjead@macalester.edu (D. Chatterjea).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.074

(i.pl.) treatments with compound 48/80 (c48/80; 0.3 μg or 1.5 μg/paw; 10 μl) or saline alone [15]. Sodium cromoglycate (SCG; 80 mg/kg), diphenhydramine (H1R antagonist; 20 mg/kg), thioperamide maleate (H3R/H4R antagonist, 10 mg/kg), or 100 μl vehicle was injected intraperitoneally (i.p.) one hour prior [16,17], and fucoidan (20 mg/kg; 200 μl) or vehicle was administered retro-orbitally (r.o.) 30 min prior to c48/80 injection [18].

### 2.3. Behavioral testing

To assess thermal sensitivity single mice treated with i.pl. c48/80 or vehicle were placed in a Plexiglas cylinder on a hotplate analgesia meter (Harvard Laboratories, Edenbridge, KY) maintained at 51.0 ± 0.5 °C for ND4 Swiss mice and 53.0 ± 0.5 °C for C57/BL6 mice and removed when prolonged retraction, flipping/licking of the hindpaw, or jumping with both hindpaws off the hotplate were observed, but no later than 40 s (adapted from [19]). Two baseline hotplate latencies were taken 24 and 48 h before the experiment. Mice with >10 s differences between baselines or <15 s averages were excluded. Nociceptive behavior was quantified by subtracting the mean baseline thermal latency from the experimental thermal latency at each time point for each mouse.

Mechanical sensitivity was measured with an Electronic von Frey Anesthesiometer (IITC Corporation, Woodland Hills, CA) as the pressure required to evoke either sharp retraction of the hindpaw, jumping with all four paws, or licking of the stimulated hindpaw [20]. Baseline latencies were calculated as the mean of the three readings closest to the median out of five readings taken 24 and 48 h before the experiment. Experimental measurements were calculated as the average of 3–4 readings per mouse without exclusions. Average baseline was subtracted from average experimental withdrawal threshold to calculate the delta withdrawal threshold for each mouse. All behavioral experiments used a minimum of 10 mice per treatment group.

### 2.4. Paw edema measurements

Change in hindpaw width measured using digital calipers (±0.1 mm; VWR) was calculated as an average of the left and right paw widths. Baseline paw widths for each mouse were taken pre-treatment and subtracted from post-treatment paw widths to calculate tissue edema.

### 2.5. Quantification of myeloperoxidase activity

Excised footpads were frozen at −80 °C in 50 mM $K_2HPO_4$ buffer (pH 6.0) containing 0.05% hexadecyl trimethylammonium bromide (HTAB), thawed, homogenized in 5× volumes of HTAB buffer,

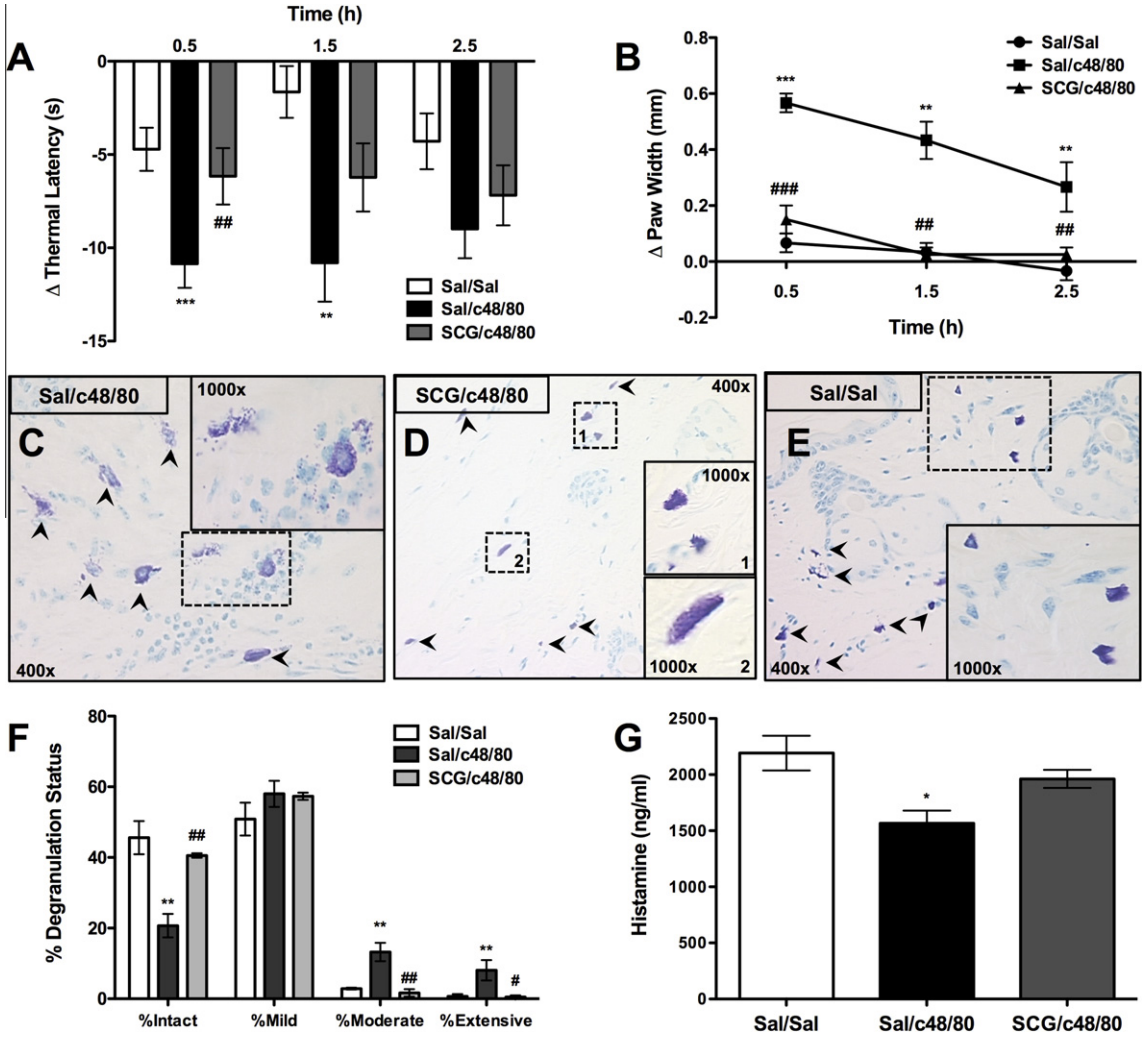


**Fig. 1.** Compound 48/80-induced plantar mast cell degranulation causes thermal hyperalgesia in mice. Compound 48/80 (0.3 μg/paw)-treated plantar tissue shows increased thermal hyperalgesia (A), edema (B), histological evidence of mast cell degranulation (C–E; 4 μm; Tb, 400×), and extent of degranulation (F) compared to saline controls and SCG-pretreated tissue (80 mg/kg i.p.). Tissue histamine levels are lower in c48/80-treated paws than saline or SCG pre-treated paws (G). * significant compared to Sal/Sal; # significant compared to Sal/c48/80. $n$ = 3–10 mice per treatment group; data represent ⩾3 separate experiments.

sonicated 3× for 10 s, freeze–thawed 3×, re-sonicated, and centrifuged for 4 min at 4750 rpm (adapted from [21,22]). Absorbance was recorded at 450 nm after a 20-min incubation in 50 mM phosphate buffer (pH 6.0) with 0.025% hydrogen peroxide and 0.167 mg/ml *o*-dianisidine dihydrochloride at room temperature in the dark. Myeloperoxidase (MPO) levels were normalized to tissue weight and presented as OD/g of wet tissue.

### 2.6. Quantification of tissue mast cells and neutrophils

Excised hindpaws were fixed in 10% buffered formalin for 24 h, transferred to 70% ethanol, decalcified for 1–2 weeks in 15% EDTA, hydrated, and embedded in paraffin. Four micrometers sagittal sections were stained with Toluidine blue (Tb) and Hematoxylin/Eosin (H&E) for quantification of mast cells and neutrophils, respectively, at 400× magnification with ⩾3 biological replicates per treatment. Tb-stained mast cells were counted in 10 fields/section and degranulation was scored based on the number of granules observed outside the boundary of the cell: intact (0), mild (1–10), moderate (10–20), and extensive (20+). Neutrophils were counted in each of 10 fields/section. Researchers performing cell counts remained blind to treatment.

### 2.7. Quantification of plantar histamine

Excised hindpaws were flash-frozen in liquid nitrogen and homogenized in Cell Lysis Buffer (Cell Signaling Technology, Beverly, MA) supplemented with protease inhibitor (Cocktail Set IV; EMD Biosciences, Billerica, MA) using a Tissue Tearor (BioSpec; Model 985370). Homogenates were incubated on ice for 20 min, centrifuged at 2000 rpm for 10 min at 4 °C, and lysates stored at −80 °C. Histamine levels were quantified by ELISA according to manufacturers' (Neogen Corporation, Lansing, MI) instructions.

### 2.8. Statistical analysis

Data were processed using Microsoft Excel and graphed with Graphpad PRISM 5.0 (San Diego, CA); statistical analyses were performed using JMP 9.0 (SAS; Cary, NC). All data are presented as the mean ± SEM, analyzed by one-way ANOVA and the Tukey–Kramer HSD post hoc test at each timepoint. Statistical significance was defined as $p < 0.05$, and denoted in the figures as: $p < 0.05$ [∗, #], $p < 0.01$ [∗∗, ##], $p < 0.001$ [∗∗∗, ###].

## 3. Results

### 3.1. Compound 48/80-mediated plantar mast cell degranulation causes thermal hyperalgesia and tissue edema

ND4 mice treated with bilateral intraplantar injections of mast cell secretagogue c48/80 (0.3 μg/paw) were significantly more sensitive to thermal stimuli compared to saline-treated mice at 0.5 and 1.5 h (Fig. 1A). The difference between latencies of saline- and c48/80-treated mice was resolved by 2.5 h post-treatment (Fig. 1A). Intraperitoneal pre-treatment with 80 mg/kg of mast cell granule stabilizer SCG blocked the early induction of nociceptive behavior at 0.5 h post intraplantar c48/80 treatment, suggesting that inhibiting mast cell degranulation abrogates thermal hyperalgesia. SCG pre-treatment alone did not result in significantly longer thermal latencies compared to saline-treated controls at any time point, thus SCG did not have a thermal analgesic effect independent of blocking degranulation (Supplementary Fig. 1). Mice treated with c48/80 had significant paw edema compared to saline-treated mice at 0.5 h after treatment; edema was maintained through 2.5 h post-treatment (Fig. 1B) and was blocked at all time points by SCG.

We observed increased mast cell degranulation in histological sections of hindpaws injected with c48/80 (Fig. 1C) but not in hindpaws of mice given SCG prior to intraplantar c48/80 (Fig. 1D) or in saline-treated paws (Fig. 1E). ∼50% of the mast cells were intact and 50% mildly degranulated in saline-treated paws (Fig. 1F). With c48/80 treatment, intact mast cells were reduced to 20% with 8–10% of mast cells showing moderate or extensive levels of degranulation. SCG pre-treatment blocked moderate and extensive degranulation and restored intact cell counts to control levels (Fig. 1F). The front paws of mice injected with either saline or

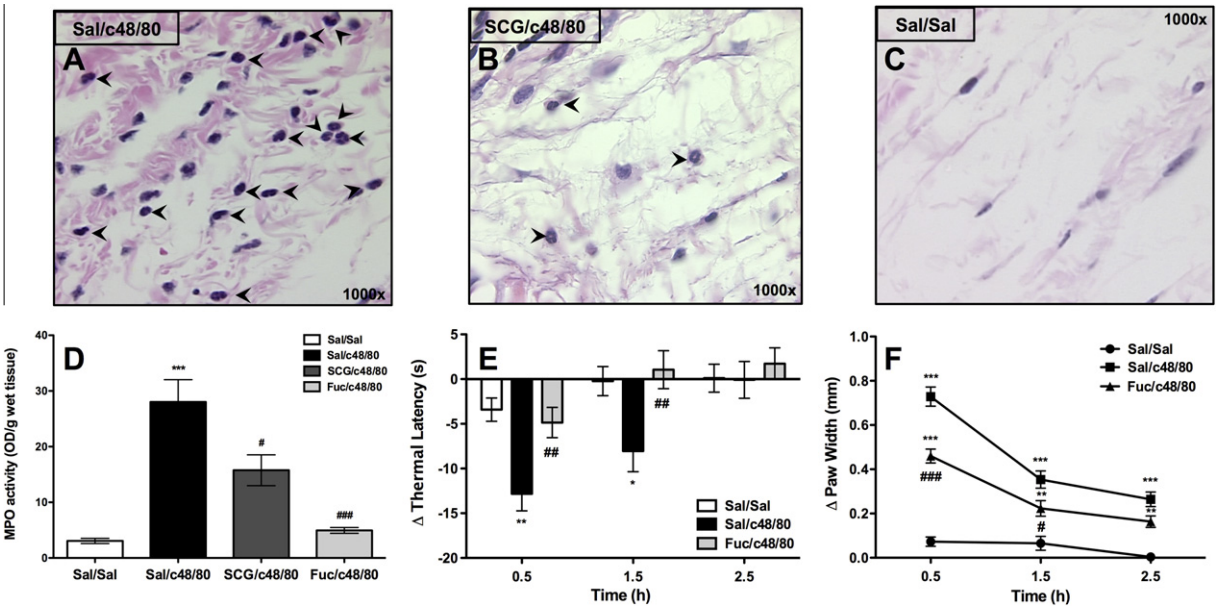


**Fig. 2.** Neutrophil infiltration caused by c48/80-induced mast cell degranulation mediates the hyperalgesic response. Compound 48/80 (1.5 μg/paw i.pl.) treated paws contain more neutrophils (A–C) and show higher MPO activity (D) than saline-treated or SCG (80 mg/kg i.p.) pre-treated paws. Fucoidan (20 mg/kg i.v.) pre-treatment reduces c48/80-mediated plantar MPO levels (D) and significantly blocks thermal hyperalgesia (E) and edema (F). * significant compared to Sal/Sal; # significant compared to Sal/c48/80. $n = 3$–10 mice per treatment group; data represent ⩾3 separate experiments.

c48/80 in the hindpaw showed no difference in levels of degranulation (Supplementary Fig. 2), demonstrating that intraplantar c48/80 administration had a strictly local effect. Tissue enzymes quickly degrade histamine released from mast cell granules leading to a reduction in histamine levels after mast cell degranulation [23]. Compound 48/80 treatment caused a 30% decrease in tissue histamine levels that was largely restored by SCG pre-treatment to stabilize mast cell granules (Fig. 1G).

### 3.2. Local neutrophil infiltration caused by c48/80-induced mast cell degranulation mediates the hyperalgesic response

H&E-stained histological sections showed clear evidence of neutrophil infiltration in c48/80-treated but not SCG pre-treated paws compared to controls (Fig. 2A–C). Levels of MPO, an enzyme frequently used to quantify neutrophil infiltration [21,22], were significantly elevated in the plantar tissue following c48/80 administration; this increase was reduced partially but significantly by pre-treatment with SCG (Fig. 2D).

We blocked neutrophil influx into the plantar tissue by systemic pre-treatment with 20 mg/kg fucoidan, an agent known to inhibit selectin-mediated leukocyte infiltration [24]. There was a marked reduction in plantar MPO activity in fucoidan pre-treated mice (Fig. 2D), which was confirmed by neutrophil counts in histological sections (Supplementary Fig. 3). Inhibition of neutrophil influx into the plantar tissue significantly reduced c48/80-induced thermal hyperalgesia at 0.5 and 1.5 h post-treatment (Fig. 2E) and partially abrogated tissue edema at 0.5 h (Fig. 2F). Mice treated with fucoidan alone did not show significantly longer thermal latencies compared to saline controls, indicating the absence of an analgesic effect independent of neutrophil blockade (Supplementary Fig. 1).

### 3.3. Histamine receptor antagonism abrogates c48/80-induced thermal hyperalgesia

Blockade of histamine signaling can abrogate thermal and mechanical hypernociception in rodents [4,25]. We found that intraperitoneal pre-treatment with H1R and H3R/H4R antagonists, diphenhydramine and thioperamide, respectively, significantly reduced c48/80-induced thermal hyperalgesia 0.5 h after plantar c48/80 challenge (Fig. 3A), partially blocked tissue edema (Fig. 3B), and reduced tissue MPO activity (Fig. 3C). We confirmed that neither diphenhydramine nor thioperamide administration caused drowsiness as neither drug significantly changed cage-crossing activity 0–4 h post-treatment (data not shown).

We observed that diphenhydramine, but not thioperamide, on its own, caused mice to remain on the hotplate longer than counterparts injected with saline alone (Supplementary Fig. 1), suggesting that the analgesic effect of diphenhydramine is only partially dependent on its effects on mast cell-derived histamine signaling.

### 3.4. Plantar mast cell reconstitution restores hyperalgesia and tissue edema in genetically mast cell-deficient C57BL/6 Wsh/Wsh mice

As neither c48/80 nor SCG acts exclusively on mast cells [26,27], we asked whether c48/80-mediated hyperalgesic responses were abrogated in genetically mast cell-deficient Wsh/Wsh mice [13] and whether hyperalgesia could be restored with tissue-specific mast cell reconstitution. We adapted our protocol to the C57BL/6 background by adjusting the c48/80 dose to 1500 ng/paw and the observation period to 4 h.

Thermal hyperalgesia was markedly decreased in mast cell-deficient Wsh/Wsh mice compared to WT mice at 1.5–4 h post-treatment (Fig. 4A). Compound 48/80-challenged Wsh/Wsh:WT mice engrafted with $2 \times 10^6$ WT cultured mast cells in the hindpaws had significantly lower delta latencies compared to non-transplanted counterparts (Fig. 4A). As hypoalgesic responses to vertically applied punctate thermal stimuli have been reported in kit-mutant mice [28], we compared baselines to confirm that Wsh/Wsh mice were at least as sensitive to the thermal stimulus applied across all four paws as WT mice (Supplementary Fig. 4).

We independently confirmed mast cell-dependent restoration of hyperalgesia with mechanical sensitivity measurements. Mechanical pain responses following c48/80 challenge were also significantly abrogated in Wsh/Wsh mice compared to their WT counterparts and restored to WT levels upon plantar mast cell reconstitution (Fig. 4B). Both thermal and mechanical latencies of c48/80-treated WT C57BL/6 were significantly lower than saline controls (data not shown).

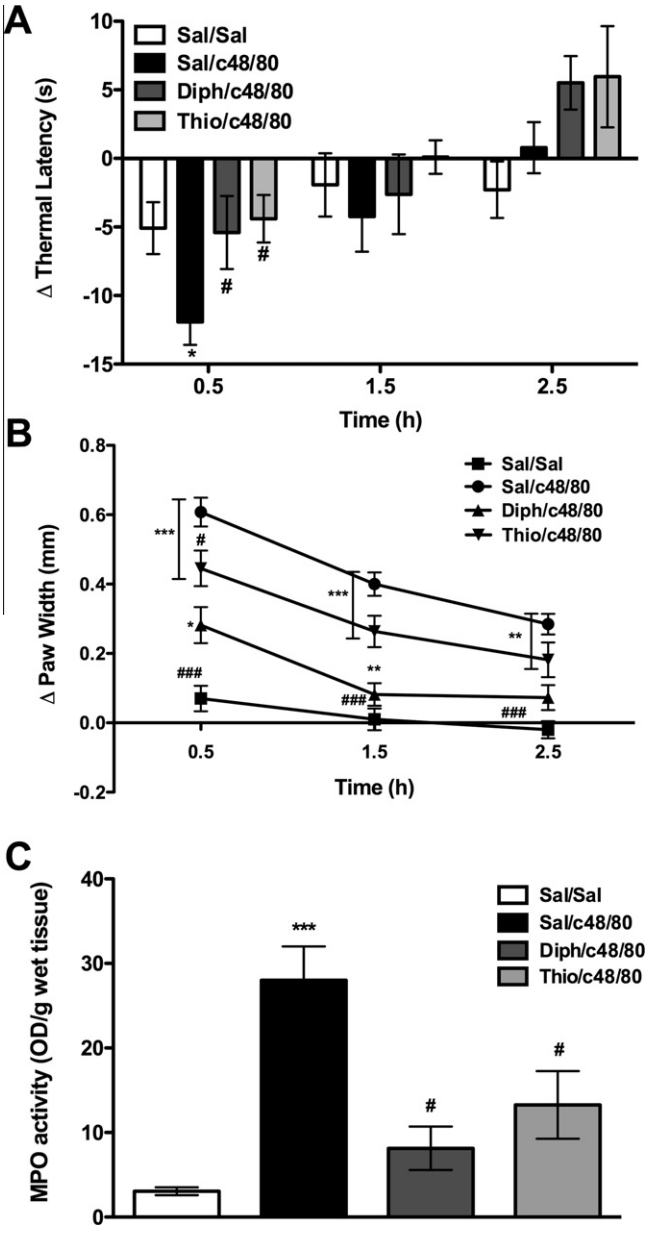


**Fig. 3.** Histamine receptor antagonism abrogates c48/80-induced thermal hyperalgesia. ND4 mice pre-treated with diphenhydramine (20 mg/kg) and thioperamide (10 mg/kg) intraperitoneally 1hour prior to c48/80 (1.5 μg/paw i.pl.) have reduced thermal hyperalgesia (A) and partially reduced hindpaw edema (B). Both pre-treatments reduced MPO activity at 2.5 h post-treatment (C). * significant compared to Sal/Sal; # significant compared to Sal/c48/80. $n$ = 3–10 mice per treatment group; data represent ⩾3 separate experiments.

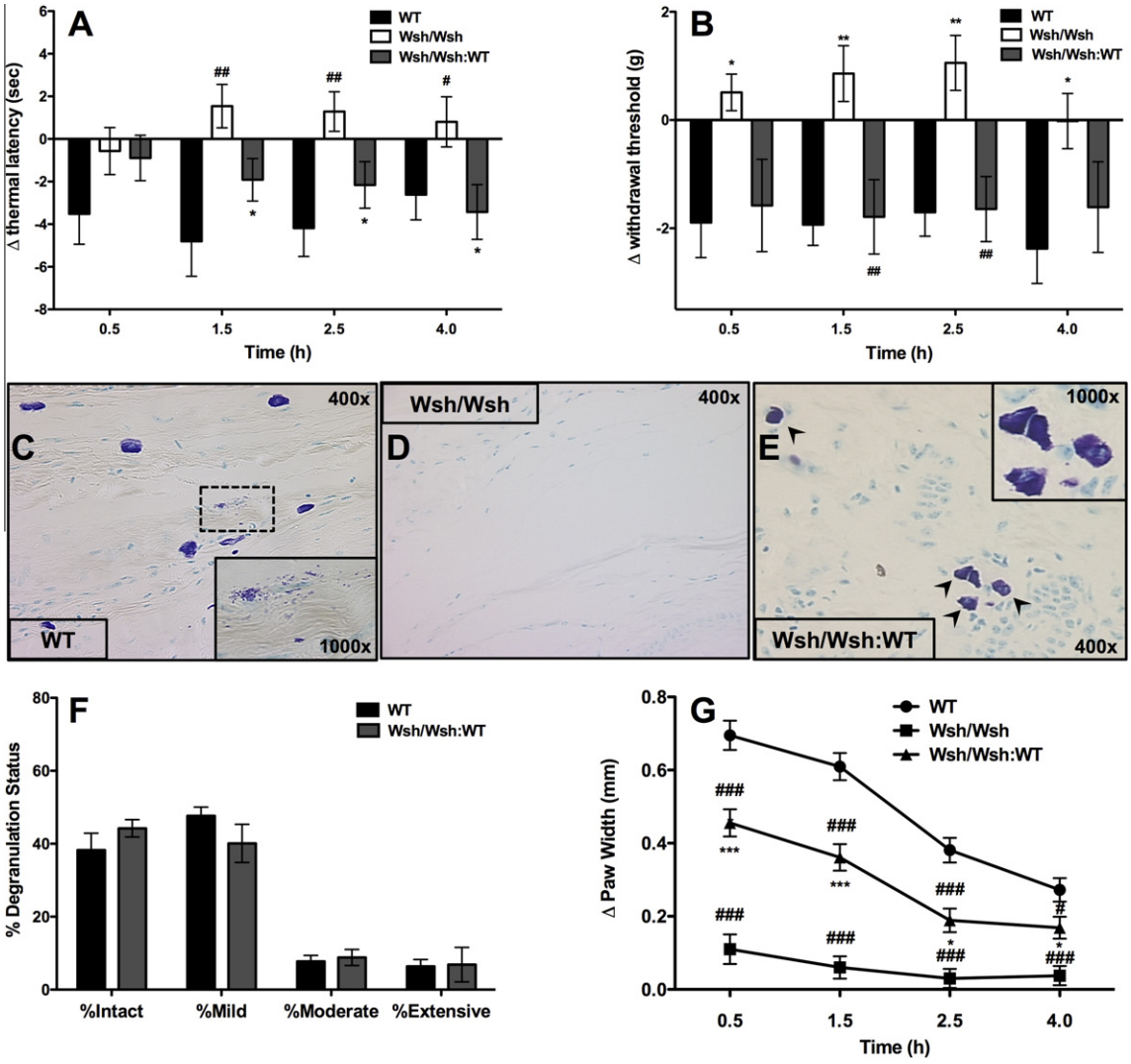


**Fig. 4.** Hyperalgesia and paw edema induced by c48/80-mediated mast cell degranulation in WT C57BL/6 mice are abrogated in Wsh/Wsh mice and restored with plantar mast cell reconstitution. Thermal (A) and mechanical (B) hyperalgesia induced by intraplantar c48/80 (1.5 μg/paw) was absent in Wsh/Wsh mice and restored in Wsh/Wsh:WT mice engrafted with plantar mast cells. Cutaneous mast cells are present in Tb-stained sections of WT and Wsh/Wsh:WT but not in Wsh/Wsh hindpaws (C–E). WT and reconstituted Wsh/Wsh:WT mice have comparable levels of mast cell degranulation following c48/80 administration (F). Hindpaw edema is reduced in Wsh/Wsh mice and partially restored in Wsh/Wsh:WT mice (G). * Significant compared to WT c48/80; # significant compared to Wsh/Wsh c48/80; + significant compared to WT saline. $n = 3–10$ mice per treatment group; data represent $\geqslant 3$ separate experiments.

**Table 1**
Number of mast cells and neutrophils in histological sections of wild-type, mast cell-deficient, and mast cell-reconstituted hindpaws after treatment with c48/80.

| | WT | Wsh/Wsh | Wsh/Wsh:WT |
|---|---|---|---|
| Mast cells/Paw | 158.0 ± 18.0 | 0.0 ± 0.0* | 132.0 ± 29.0# |
| Neutrophils/Paw | 113.8 ± 20.9 | 20.7 ± 0.9* | 81.5 ± 19.5# |

* Significant compared to WT.
\# Significant compared to Wsh/Wsh.

Mast cells were present in representative sections of plantar tissue from WT and Wsh/Wsh:WT mice but not in Wsh/Wsh mice (Fig. 4C–E); absolute counts confirmed that plantar mast cells were reconstituted to approximately 70% of WT levels in mast cell-reconstituted paws (Table 1). Neutrophil influx was restored to about 70% of WT levels in histological sections of mast cell-reconstituted mice (Table 1). We found comparable levels ($p > 0.05$) of mild, moderate, and extensive degranulation of mast cells in WT and mast cell-reconstituted Wsh/Wsh:WT mice (Fig. 4F). Compound 48/80-induced tissue edema was absent in Wsh/Wsh mice but restored to approximately half of WT levels in plantar mast cell-reconstituted mice (Fig. 4G).

Taken together, these data showed that c48/80-induced pain and edema responses could be depleted and restored in a mast cell-dependent manner.

## 4. Discussion

Here we show that stabilization of plantar mast cell granules limits c48/80-mediated hindpaw hyperalgesia and edema in ND4 mice, demonstrating that mast cell degranulation can initiate inflammatory pain. Systemic mast cell degranulation can cause cranial, tailbase, and hindpaw sensitivity in rats through central sensitization mechanisms [12]. Our findings indicate that localized mast cell activation in the hindpaw leads to pain responses via local neutrophil influx and histamine release. Taken together, these two studies provide strong evidence that mast cells contribute to both central and peripheral pain pathways in rodents.

Recruitment of neutrophils into inflamed tissue has been shown to be dependent upon mast cell activation in host defense against bacterial infection [29] and collagen-induced arthritis, [30]. Neutrophils have been previously shown to mediate carrageenan-induced mechanical hyperalgesia in mice [18] and allergen-evoked thermal hyperalgesia in rats [25]. In our study, c48/80-mediated neutrophil infiltration into the hindpaw tissue of ND4 mice was partially, but significantly, inhibited by mast cell granule stabilization with SCG pre-treatment. Fucoidan pre-treatment blocked neutrophil influx and significantly reduced thermal hyperalgesia in c48/80-treated ND4 mice. Compound 48/80-mediated mast cell degranulation also decreased the total histamine content of the hindpaw, consistent with the rapid degradation of free histamine by tissue enzymes [23]. Histamine signaling has been shown to mediate formalin- and antigen-induced pain and sensitize meningeal nociceptors [25,31]. Here, pre-treatment with H3R/H4R dual antagonist thioperamide attenuated c48/80-induced thermal pain 30 min after challenge without causing analgesia when administered alone, implicating H3R and H4R in pro-nociceptive mast cell signaling. H3R is primarily located in the central nervous system and is not accessible by histamine released locally in the hindpaw [32]. H4R has been shown to modulate mast cells, eosinophils, T cells, and dendritic cells [32], as well as carrageenan- and Complete Freund's Adjuvant-induced mechanical hyperalgesia in rats [33]. H4R, therefore, may be the primary receptor involved in the early pro-nociceptive signaling in the periphery. We found that H4R (and H1R) antagonism significantly reduced the infiltration of neutrophils into c48/80-treated paws, supporting earlier observations that H4R blockade prevents neutrophil influx in zymosan peritonitis and carrageenan-treated plantar tissue [33]. Mast cell degranulation-initiated pain, therefore, is crucially mediated through histamine signaling and neutrophil influx.

Interestingly, our findings suggest that pain and edema resulting from c48/80-induced mast cell degranulation may, in part, be differentially regulated. While SCG pre-treatment reduced pain and edema responses, H3/4R antagonist thioperamide blocked thermal pain to control levels but only partially reduced edema. Blocking neutrophil influx, similarly, markedly reduced pain responses but only partially blocked tissue edema. In a rat model of allergen-evoked pain, bradykinin and histamine receptor antagonists can alleviate pain but not edema [25]. Mast cell deficiency can inhibit hyperalgesia, but not vasodilation, in murine interstitial cystitis [4]. Our findings support independent regulation of pain and edema despite their co-occurrence in inflammatory cascades.

We found that genetically mast cell-deficient Wsh/Wsh mice have reduced thermal and mechanical pain and edema, and local plantar reconstitution with bone marrow-derived mast cells can restore these responses. While Rudick et al. found that whole bone marrow transplantation restored cystitis pain in Wsh/Wsh mice [4], their study did not specifically isolate mast cells as the relevant cellular players. Repair of other c-Kit-related defects may have played unspecified roles. We provide the first evidence that tissue-specific reconstitution of mast cells restores the c48/80-induced thermal and mechanical pain responses abrogated in mast cell-deficient mice. Future experiments in recently described kit-independent mouse strains [34] with conditional mast cell deficiencies will further elucidate contributions of these effectors to pain responses in the absence of systemic c-Kit defects.

As mast cells are the primary source of histamine in the tissue, we were not surprised to find virtually no detectable histamine in the hindpaws of Wsh/Wsh mice (data not shown). Furthermore, mast cell-deficient Wsh/Wsh mice showed significantly decreased neutrophil numbers in the hindpaw after c48/80-injection, and neutrophil infiltration was partially, but significantly, restored with plantar mast cell reconstitution. These findings demonstrate that the contributions of histamine signaling and neutrophil influx to c48/80-mediated inflammatory pain are significant and mast cell-dependent.

In recent years, mast cell contributions to human pain pathologies have been explored. Cutaneous administration of c48/80 to ten healthy men caused increased thermal sensitivity after two consecutive doses at 0 and 3 h, while continued administration of c48/80 at 3-h intervals over 24 h decreased subjects' thermal sensitivity and hyperalgesic response to capsaicin [8]. The early response seen in this study is consistent with the rapid thermal hyperalgesia we observed in mice, and the later response could reflect the gradual depletion of mast cell mediators after chronic exposure to c48/80. Mast cells have been implicated in vulvodynia and vestibulitis with increased numbers of degranulated mast cells in vulvar biopsies of patients [35]. Increased levels of mast cell tryptase are found in prostatic secretions of men with pelvic pain [36]. Additionally, increased dermal mast cell degranulation co-occurs with increased sensitivity to heat and pressure in children with self-injurious behaviors [37]. Here we have demonstrated that mast cells are necessary for c48/80-induced inflammatory pain responses in mice. Tissue-specific mast cell reconstitution restores neutrophil and histamine-mediated, c48/80-induced, localized hyperalgesia in mast cell-deficient mice. Our findings contribute to a growing body of literature that establishes mast cells as important and necessary players and therapeutic targets in the initiation of inflammatory pain responses.

## 5. Funding

DC is supported by NIH-AREA R15 NS067536-01A.

## Acknowledgments

We thank Dr. Stephen Galli (Stanford University) for Wsh/Wsh and reconstituted mice, the University of Minnesota Comparative Pathology Shared Resource for histology services, Dr. Lin Aanonsen for helpful discussions, and Grace Linder, Paul Maitland-McKinley, Estela Shabani, Akilah Sykes, and Robin Willenbring in our laboratory for their assistance.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.074.

## References

[1] K. Ren, R. Dubner, Interactions between the immune and nervous systems in pain, Nat. Med. 16 (2010) 1267–1276.
[2] S.J. Galli, M. Tsai, Mast cells in allergy and infection: versatile effector and regulatory cells in innate and adaptive immunity, Eur. J. Immunol. 40 (2010) 1843–1851.
[3] D. Levy, R. Burstein, V. Kainz, et al., Mast cell degranulation activates a pain pathway underlying migraine headache, Pain 130 (2007) 166–176.
[4] C.N. Rudick, P.J. Bryce, L.A. Guichelaar, et al., Mast cell-derived histamine mediates cystitis pain, PLoS ONE 3 (2008) e2096.
[5] S.M. Oliveira, C.C. Drewes, C.R. Silva, et al., Involvement of mast cells in a mouse model of postoperative pain, Eur. J. Pharmacol. 672 (2011) 88–95.
[6] G. Barbara, V. Stanghellini, R. De Giorgio, et al., Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome, Gastroenterology 126 (2004) 693–702.
[7] V. Anaf, C. Chapron, I. El Nakadi, et al., Pain, mast cells, and nerves in peritoneal, ovarian, and deep infiltrating endometriosis, Fertil. Steril. 86 (2006) 1336–1343.
[8] P.D. Drummond, The effect of cutaneous mast cell degranulation on sensitivity to heat, Inflamm. Res. 53 (2004) 309–315.
[9] R.A. Ribeiro, M.L. Vale, S.M. Thomazzi, et al., Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice, Eur. J. Pharmacol. 387 (2000) 111–118.
[10] C.A. Parada, C.H. Tambeli, F.Q. Cunha, et al., The major role of peripheral release of histamine and 5-hydroxytryptamine in formalin-induced nociception, Neuroscience 102 (2001) 937–944.

[11] T. Liu, Z.T. Bai, X.Y. Pang, et al., Degranulation of mast cells and histamine release involved in rat pain-related behaviors and edema induced by scorpion Buthus martensi Karch venom, Eur. J. Pharmacol. 575 (2007) 46–56.
[12] D. Levy, V. Kainz, R. Burstein, et al., Mast cell degranulation distinctly activates trigemino-cervical and lumbosacral pain pathways and elicits widespread tactile pain hypersensitivity, Brain Behav. Immun. 26 (2012) 311–317.
[13] M.A. Grimbaldeston, C.C. Chen, A.M. Piliponsky, et al., Mast cell-deficient W-sash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo, Am. J. Pathol. 167 (2005) 835–848.
[14] N. Depinay, F. Hacini, W. Beghdadi, et al., Mast cell-dependent down-regulation of antigen-specific immune responses by mosquito bites, J. Immunol. 176 (2006) 4141–4146.
[15] A.P. Piovezan, P. D'Orléans-Juste, M. Frighetto, et al., Endothelins contribute towards nociception induced by antigen in ovalbumin-sensitised mice, Br. J. Pharmacol. 141 (2004) 755–763.
[16] E.B. Nascimento Jr., K.A. Costa, C.M. Bertollo, et al., Pharmacological investigation of the nociceptive response and edema induced by venom of the scorpion *Tityus serrulatus*, Toxicon 45 (2005) 585–593.
[17] D. Farzin, F. Nosrati, Modification of formalin-induced nociception by different histamine receptor agonists and antagonists, Eur. Neuropsychopharmacol. 17 (2007) 122–128.
[18] T.M. Cunha, W.A. Verri Jr., I.R. Schivo, et al., Crucial role of neutrophils in the development of mechanical inflammatory hypernociception, J. Leukoc. Biol. 83 (2008) 824–832.
[19] R. Groth, L. Aanonsen, Spinal brain-derived neurotrophic factor (BDNF) produces hyperalgesia in normal mice while antisense directed against either BDNF or trkB, prevent inflammation-induced hyperalgesia, Pain 100 (2002) 171–181.
[20] G.G. Vivancos, W.A. Verri Jr., T.M. Cunha, et al., An electronic pressure-meter nociception paw test for rats, Braz. J. Med. Biol. Res. 37 (2004) 391–399.
[21] P.P. Bradley, D.A. Priebat, R.D. Christensen, et al., Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker, J. Invest. Dermatol. 78 (1982) 206–209.
[22] M. Asano, S. Nakae, N. Kotani, et al., Impaired selectin-ligand biosynthesis and reduced inflammatory responses in beta-1,4-galactosyltransferase-I-deficient mice, Blood 102 (2003) 1678–1685.
[23] W. Feldberg, J. Talesnik, Reduction of tissue histamine by compound 48/80, J. Physiol. 120 (1953) 550–568.
[24] M.E. Preobrazhenskaya, A.E. Berman, V.I. Mikhailov, et al., Fucoidan inhibits leukocyte recruitment in a model peritoneal inflammation in rat and blocks interaction of P-selectin with its carbohydrate ligand, Biochem. Mol. Biol. Int. 43 (1997) 443–451.
[25] T.R. Lavich, R.S. Cordeiro, J.B. Calixto, et al., Combined action of vasoactive amines and bradykinin mediates allergen-evoked thermal hyperalgesia in rats, Eur. J. Pharmacol. 462 (2003) 185–192.
[26] V.A. Palomäki, J.T. Laitinen, The basic secretagogue compound 48/80 activates G proteins indirectly via stimulation of phospholipase D-lysophosphatidic acid receptor axis and 5-HT1A receptors in rat brain sections, Br. J. Pharmacol. 147 (2006) 596–606.
[27] M. Dixon, D.M. Jackson, I.M. Richards, The action of sodium cromoglycate on 'C' fibre endings in the dog lung, Br. J. Pharmacol. 70 (1980) 11–13.
[28] N. Milenkovic, C. Frahm, M. Gassmann, et al., Nociceptive tuning by stem cell factor/c-Kit signaling, Neuron 56 (2007) 893–906.
[29] R. Malaviya, T. Ikeda, E. Ross, et al., Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha, Nature 381 (1996) 77–80.
[30] T.A. Pimentel, A.L. Sampaio, F. D'Acquisto, et al., An essential role for mast cells as modulators of neutrophils influx in collagen-induced arthritis in the mouse, Lab. Invest. 91 (2011) 33–42.
[31] H.A. Ashmawi, L.M. Braun, A.M. Sousa, et al., Analgesic effects of H1 receptor antagonists in the rat model of formalin-induced pain, Rev. Bras. Anestesiol. 59 (2009) 461–470.
[32] C.A. Akdis, K. Blaser, Histamine in the immune regulation of allergic inflammation, J. Allergy Clin. Immunol. 112 (2003) 15–22.
[33] G.C. Hsieh, P. Chandran, A.K. Salyers, et al., H4 receptor antagonism exhibits anti-nociceptive effects in inflammatory and neuropathic pain models in rats, Pharmacol. Biochem. Behav. 95 (2010) 41–50.
[34] H.R. Katz, K.F. Austen, Mast cell deficiency, a game of kit and mouse, Immunity 35 (2011) 668–670.
[35] J. Bornstein, N. Goldschmid, E. Sabo, Hyperinnervation and mast cell activation may be used as histopathologic diagnostic criteria for vulvar vestibulitis, Gynecol. Obstet. Invest. 58 (2004) 171–178.
[36] J.D. Done, C.N. Rudick, M.L. Quick, et al., Role of mast cells in male chronic pelvic pain, J. Urol. 187 (2012) 1473–1482.
[37] F.J. Symons, G. Wendelschafer-Crabb, W. Kennedy, et al., Degranulated mast cells in the skin of adults with self-injurious behavior and neurodevelopmental disorders, Brain Behav. Immun. 23 (2009) 365–370.