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

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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.

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 (2×10^6/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.

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(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,

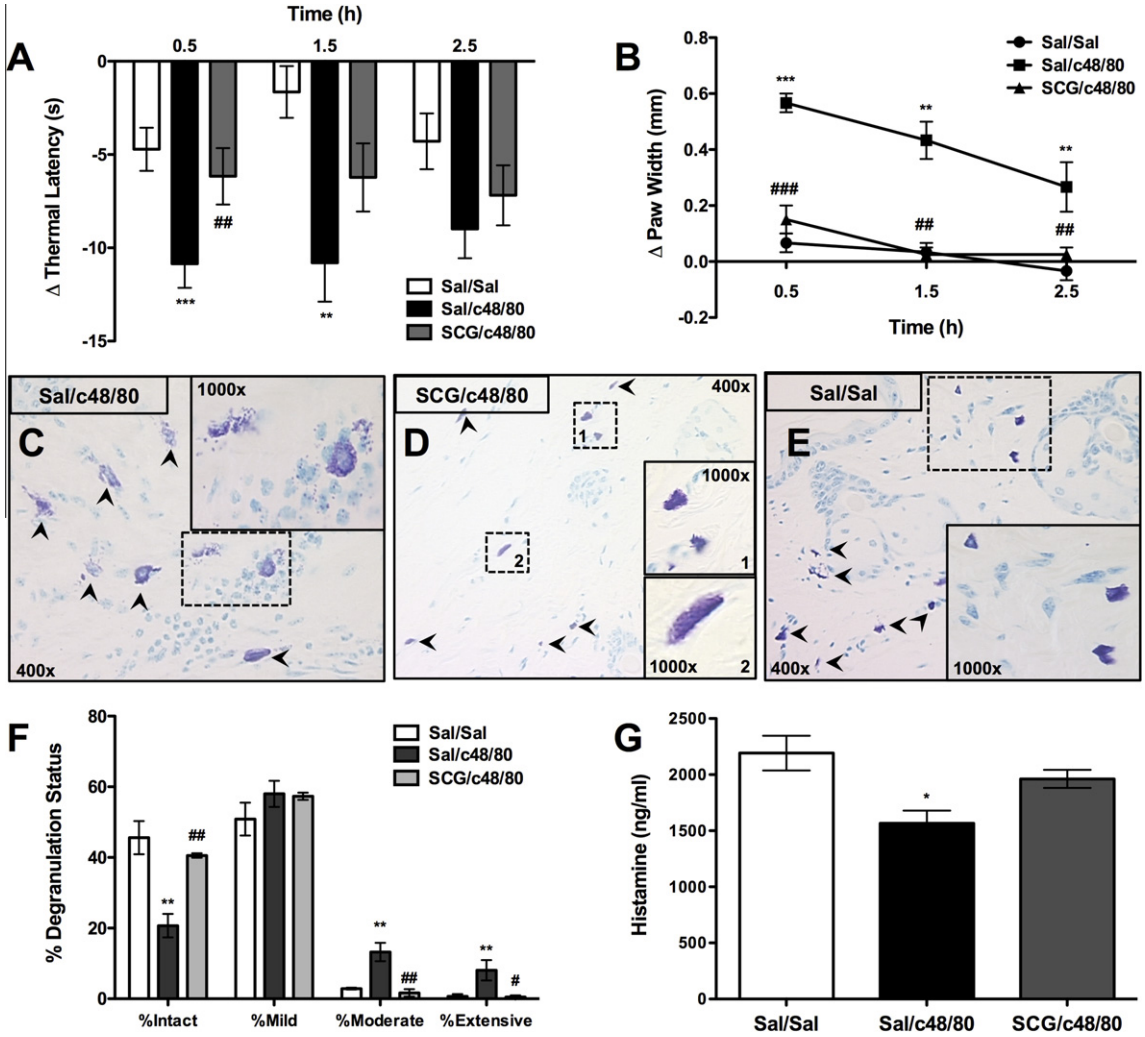

Fig. 1. Compound 48/80-induced plantar mast cell degranulation causes thermal hyperalgesia in mice. Compound 48/80 (0.3 μg/paw)-treated plantar tissue shows increased thermal hyperalgesia (A), edema (B), histological evidence of mast cell degranulation (C–E; 4 μm; Tb, 400×), and extent of degranulation (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.