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CROSS-SENSITIZATION OF HISTAMINE-INDEPENDENT ITCH IN MOUSE PRIMARY SENSORY NEURONS

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Abstract—Overexpression of pruritogens and their precursors may contribute to the sensitization of histamine-dependent and –independent itch-signaling pathways in chronic itch. We presently investigated self- and cross-sensitization of scratching behavior elicited by various pruritogens, and their effects on primary sensory neurons. The MrgprC11 agonist BAM8-22 exhibited self- and reciprocal cross-sensitization of scratching evoked by the protease-activated receptor-2 (PAR-2) agonist SLIGRL. The MrgprA3 agonist chloroquine unidirectionally cross-sensitized BAM8-22-evoked scratching. Histamine unidirectionally cross-sensitized scratching evoked by chloroquine and BAM8-22. SLIGRL unidirectionally cross-sensitized scratching evoked by chloroquine. Dorsal root ganglion (DRG) cells responded to various combinations of pruritogens and algogens. Neither chloroquine, BAM8-22 nor histamine had any effect on responses of DRG cell responses to subsequently applied pruritogens, implying that their behavioral self- and cross-sensitization effects are mediated indirectly. SLIGRL unilaterally cross-sensitized responses of DRG cells to chloroquine and BAM8-22, consistent with the behavioral data. These results indicate that unidirectional cross-sensitization of histamine-independent itch-signaling pathways might occur at a peripheral site through PAR-2. PAR-2 expressed in pruriceptive nerve endings is a potential target to reduce sensitization associated with chronic itch.

Key words: itch, pruritogen, scratching, dorsal root ganglion (DRG) cell, calcium imaging, cross-sensitization.

INTRODUCTION

Chronic itch is a burdensome clinical problem that decreases the quality of life (Weisshaar et al., 2006). The mechanisms underlying chronic itch appear to involve sensitization of itch-signaling pathways, as manifested by hyperknesis (enhanced itch) and alloknesis (touch-evoked itch) (Simone et al., 1991; Ikoma et al., 2003; Akiyama et al., 2010, 2011, 2012). These features of sensitization are observed for both histamine-dependent and -independent types of itch-related scratching behavior in animal studies (Akiyama et al., 2010, 2012), but the precise mechanisms are still unknown.

Histamine is a well-known itch mediator and is elevated in the skin of atopic dermatitis patients (Johnson et al., 1960), although its primary role in chronic itch is suspect (Heyer et al., 1989, 1998). Recent studies reveal two distinct histamine-independent itch pathways that are mediated by Mas-related G protein-coupled receptors (Mrgprs) and protease-activated receptor type-2 (PAR-2) (Steinhoff et al., 2003; Liu et al., 2009). Mrgprs consist of over 50 members that include MrgprAs, MrgprB4-5, MrgprC11 and MrgprD and are restricted to small diameter dorsal root ganglion (DRG) neurons in mice (Dong et al., 2001). Chloroquine and the bovine adrenal medulla peptide 8-22 (BAM8-22) elicit itch-related scratching through MrgprA3 and MrgprC11, respectively, in mice (Liu et al., 2009) and BAM8-22 elicits itch in humans (Sikand et al., 2011). Expression of the precursor of BAM8-22, proenkephalin A, in skin is increased under pathological conditions including psoriasis (Slominski et al., 2011). Another histamine-independent itch pathway is PAR-2, which is a member of the protease-activated receptor family. Both endogenous and exogenous agonists of PAR-2, tryptase and SLIGRL-NH2, respectively, elicit scratching in a dose-dependent manner (Shimada et al., 2006; Ui et al., 2006; Akiyama et al., 2009). The level of PAR-2 expression in nerves is increased in the skin of atopic dermatitis patients (Steinhoff et al., 2003). Furthermore, the expression levels of endogenous PAR-2 agonists including tryptase and kallikreins are elevated in the skin of atopic dermatitis patients (Steinhoff et al., 2003; Komatsu et al., 2007). The aim of this study was to test if histamine and various non-histamine itch mediators interact and contribute to sensitization of itch signaling.

EXPERIMENTAL PROCEDURE

Behavioral scratching studies

Experiments were conducted using 42 adult male C57BL/6 mice (Simonsen, Gilroy, CA, USA; 19–26 g) under a protocol approved by the UC Davis Animal Care and Use Committee.

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Abbreviations: 5-HT, 5-hydroxytrmptamine; AITC, allyl isothiocyanate; ANOVA, analysis of variance; BAM8-22, bovine adrenal medulla peptide 8-22; CQ, chloroquine; DRG, dorsal root ganglion; Mrgpr, Mas-related G protein-coupled receptor; PAR, protease-activated receptor; SLIGRL-NH2, PAR-2 agonist.

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Table 1. Experimental groups to investigate self-sensitization, cross-sensitization in scratching behavior. Column denoted "first" indicates which drug was applied first, and column denoted "second" indicates which agent (if any) was applied next in the same session. After the first agent was injected, mice were videotaped for 40 min, at which time a second injection of the same or a different mediator was made at the same site and mice were videotaped for another 40 min. All treatments are intradermal

Group	First	Dose/Route	Second	Dose/Route
1	Chloroquine	193 nmol/10 μl	Chloroquine	193 nmol/10 μl
2	Chloroquine	193 nmol/10 μl	BAM8-22	50 nmol/10 μl
3	Chloroquine	193 nmol/10 μl	Histamine	271 nmol/10 μl
4	Chloroquine	193 nmol/10 μl	SLIGRL-NH2	76 nmol/10 μl
5	BAM8-22	50 nmol/10 μl	Chloroquine	193 nmol/10 μl
6	BAM8-22	50 nmol/10 μl	BAM8-22	50 nmol/10 μl
7	BAM8-22	50 nmol/10 μl	Histamine	271 nmol/10 μl
8	BAM8-22	50 nmol/10 μl	SLIGRL-NH2	76 nmol/10 μl
9	Histamine	271 nmol/10 μl	Chloroquine	193 nmol/10 μl
10	Histamine	271 nmol/10 μl	BAM8-22	50 nmol/10 μl
11	SLIGRL-NH2	76 nmol/10 μl	Chloroquine	193 nmol/10 μl
12	SLIGRL-NH2	76 nmol/10 μl	BAM8-22	50 nmol/10 μl

The fur on the rostral back was shaved and mice were habituated to the Plexiglas recording arena 1 wk prior to testing. On the test day, the animal received an intradermal (i.d.) microinjection of 10 μl of one of the following: nothing (pretreatment none), histamine (271 nmol; Sigma–Aldrich, St. Louis, MO, USA), PAR-2 agonist SLIGRL-NH2 (76 nmol; Quality Controlled Biochemicals, Hopkinton, MA, USA), chloroquine (193 nmol; Sigma) or BAM8-22 (50 nmol; Genemed Synthesis Inc., San Antonio, TX, USA). Microinjections were made i.d in the nape of the neck using a 30-G needle attached to a Hamilton microsyringe by PE-50 tubing. The injection site was marked so a second injection could be made at the same location in experiments testing for self-sensitization and cross-sensitization (Table 1). Immediately after the injection the mouse was placed into the arena and videotaped from above for 40–60 min. Generally 3–4 mice were injected and videotaped simultaneously. Immediately after commencing videotaping all investigators left the room.

Videotapes were reviewed by investigators blinded to the treatment, and the number of scratch bouts was counted at 5-min intervals. A scratch bout was defined as one or more rapid back-and-forth hind paw motions directed toward and contacting the injection site, and ending with licking or biting of the toes and/or placement of the hind paw on the floor. Hind paw movements directed away from the injection site (e.g., ear-scratching) and grooming movements were not counted. For analysis of the time course of scratching, two-way repeated-measures analysis of variance (ANOVA) followed by Student–Newman–Keuls test was used to compare the mean number of scratch bouts/5-min interval after pruritogen injection without vs. with pretreatment. One-way ANOVA followed by the Bonferroni post-test was used to compare the total number of scratch bouts across pretreatment groups. In all cases $p < 0.05$ was considered to be significant.

Calcium imaging

A total of 43 adult male C57/BL6 mice (7–9 wks old, 18–21 g) was used under a protocol approved by the UC Davis Institutional Animal Care and Use Committee. In one set of experiments, 13 juvenile mice (3–4 wks old, 8–12 g) were used. The animal was euthanized under sodium pentobarbital anesthesia and upper- to mid-cervical DRGs were acutely dissected and enzymatically digested at 37 °C for 10 min in Hanks's balanced salt solution (HBSS; Invitrogen, Carlsbad, CA, USA) containing 20 units/ml papain (Worthington Biochemical, Lakewood, NJ, USA) and 6.7 mg/ml L-cysteine (Sigma), followed by 10 min at 37 °C in HBSS containing 3 mg/ml collagenase (Worthington Biochemical). The ganglia were then mechanically triturated using fire-polished glass pipettes. DRG cells were pelleted, suspended in MEM Eagle's with Earle's BSS (Gibco) containing 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco), 1 × vitamin (Gibco) and 10% horse serum (Quad Five, Ryegate, MT), plated on poly-D-lysine-coated glass coverslips, and cultured for 16–24 h.

DRG cells were incubated in Ringers solution (pH7.4; 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 4.54 mM NaOH) with 10 μM of Fura-2 AM and 0.05% of Pluronic F-127 (Invitrogen). Coverslips were mounted on a custom-made aluminum perfusion block and viewed through an inverted microscope (Nikon TS100, Technical Instruments, San Francisco, CA, USA). Fluorescence was excited by UV light at 340 and 380 nm alternately and emitted light was collected via a CoolSnap camera attached to a Lambda LS lamp and a Lambda optical filter changer (Sutter Instrument Company, Novato, CA, USA). Ratiometric measurements were made using Simple PCI software (Compix Inc, Cranberry Township, PA, USA) every 3 s.

Solutions were delivered by a solenoid-controlled 8-channel perfusion system (ValveLink, AutoM8). Two of the following agents were delivered in randomized order: chloroquine (300 μM), BAM8-22 (10 μM), histamine (100 μM) and the PAR-2 agonist SLIGRL-NH2 (100 μM). This was then followed by AITC (200 μM) or capsaicin (1 μM). The concentrations selected for histamine and the PAR-2 agonist were the same as used in prior studies (histamine: Han et al., 2006; Nicolson et al., 2007; Shim et al., 2007; Akiyama et al., 2010; PAR-2 agonist: Steinhoff et al., 2000; Amadesi et al., 2004; Akiyama et al., 2010). Mrgpr agonist concentrations were selected based on pilot experiments to determine the concentration that activated the highest percentage of DRG cells. For chloroquine, the percentages of DRG cells activated were as follows: 1 mM: 8.1% (34/420), 300 μM: 9.8% (189/742), 100 μM: 1.8% (7/386). For BAM8-22, the percentages were: 10 μM: 2.1% (4/184), 3 μM: 0.1% (1/138). In the experiments testing self-sensitization, chloroquine or BAM8-22 was delivered twice at a 3-min interstimulus interval. Potassium at a concentration of 144 mM was always delivered at the end of each experiment. Stimulus duration was 30 s (10 s for capsaicin). Ratios were normalized to baseline. Cells were judged to be sensitive if the ratio value increased by more than 10% of the resting level following chemical application. Only cells responsive to high-K^+ were included for analysis. Between-group differences were compared by one-way ANOVA followed by Bonferroni's post hoc test.