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Plenary article

Enhanced scratching elicited by a pruritogen and an algogen in a mouse model of contact hypersensitivity

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HIGHLIGHTS

- Contact hypersensitivity (CHS) altered chemically evoked behaviors in mice.
- CHS enhanced itch and pain behaviors evoked by BAM8-22.
- CHS enhanced the itch but not the pain behaviors evoked by bradykinin.
- CHS had no effect on the behaviors evoked by histamine.

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ABSTRACT

Chemical pruritogens and algogens evoke primarily itch and pain, respectively, when administered to the skin of healthy human subjects. However, the dominant sensory quality elicited by an algesic chemical stimulus may change in patients with chronic itch where bradykinin, elicits itch in addition to pain. Here we tested whether normally pruritic and algesic chemicals evoked abnormal itch- or pain-like behaviors in the mouse after the development of contact hypersensitivity (CHS), an animal model of allergic contact dermatitis. Mice previously sensitized to a hapten (squaric acid dibutylester) applied to the abdomen, exhibited spontaneous itch-like scratching and pain-like wiping directed to the site on the cheek of the CHS elicited by a subsequent challenge with the same hapten. In comparison with responses of control mice, CHS mice exhibited a significant increase in the scratching evoked by bovine adrenal medulla 8-22, a peptide that elicits a histamine-independent itch, but did not alter the scratching to histamine. Bradykinin, an algogen that elicited only wiping in control mice, additionally evoked significant scratching in CHS mice. Thus, within an area of CHS, histamine-independent itch is enhanced and chemically evoked pain is accompanied by itch.

1. Introduction

Pruritic chemicals normally elicit a dominant sensation of itch and algesic chemical, pain when applied to the skin of humans and, itch-like and pain-like behaviors when applied to the cheek of the mouse [1–4]. However, these sensations and sensory behaviors are not fully consistent with the observations in patients with chronic itch or chronic neuropathic pain [5–8]. For example, bradykinin, an algogen that is normally painful and not itchy, elicited itch as well as pain when administered to lesional skin of patients with atopic dermatitis [7]. And the pruritogen, histamine, evoked pain but not itch when delivered to an area of hyperalgesia in patients with post-herpetic neuralgia [8]. These alterations in sensory qualities may result from the sensitization of neurons mediating itch or pain in the peripheral and/or central nervous system [5–10].

Allergic contact dermatitis (ACD) is often accompanied by spontaneous itch and pain [11,12]. In a recent study, human subjects, previously sensitized to the contact sensitizer squaric acid dibutylester (SADBE), reported spontaneous itch and nociceptive sensations within an area of ACD produced by a subsequent application of the chemical [11]. Within this area, heat stimuli that in

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normal skin elicit only pain sensation, elicited the additional sensation of itch and intradermal injection of certain pruritic chemicals evoked an enhanced itch [11].

SADBE was used in a similar fashion in the mouse to produce an area of CHS (model of ACD in humans) on the leg or cheek [13]. Analogous to humans reporting spontaneous itch and nociceptive sensations with ACD [11], the mouse exhibited spontaneous itch- and pain-like behaviors directed to the site of CHS [13]. Moreover, one type of cutaneous nociceptor expressing Mas-related G-protein-coupled receptor A3 (MrgprA3) exhibited electrophysiological signs of hyperexcitability in response to noxious heat or mechanical stimuli applied to their receptive fields within the area of CHS [13]. In other studies, most neurons expressing MrgprA3 were shown to respond to multiple pruritogens, including histamine and to bovine adrenal medulla 8-22 (BAM8-22), a peptide cleaved from proenkephalin A [14,15]. To our knowledge, there is little information available on the behavioral responses to pruritic or algesic chemical stimuli delivered to an area of CHS in the mouse. Our purpose was to test whether the itch- and pain-like behaviors normally elicited by an algesic or pruritic chemical are altered when the same stimuli are delivered to the site of SADBE-induced CHS on the cheek of the mouse.

2. Materials and methods

2.1. Animals

C57BL/6 mice (Charles River, Wilmington, MA), 64 males, were tested, each weighing between 20 and 25 g. Mice were housed in groups of four under a 12 h light/dark cycle. During brief anesthesia with isoflurane (2% in 100% oxygen), each cheek and the abdomen were shaved at least two days before the application of a chemical to the skin. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Yale University School of Medicine and were in accordance with the guidelines provided by the National Institute of Health and the International Association for the Study of Pain.

2.2. Chemicals

BAM8-22 was obtained from Tocris Bioscience (Ellisville, MO, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). SADBE was dissolved in a vehicle of acetone. Histamine dihydrochloride, BAM8-22 and bradykinin were each dissolved in a vehicle of sterile, normal saline. The doses of all chemicals used in this study were based on the results of pilot studies or published findings [3,4,16].

2.3. Induction of contact hypersensitivity in the mouse

CHS was produced on the mouse cheek as described [13] and schematically summarized in Fig. 1. The mice were sensitized by the topical application of 25 μL of 1% SADBE in acetone to abdominal skin once daily for three consecutive days. Five days later, the right cheek was challenged either with a topical application of 25 μL of 1% SADBE in acetone ("CHS mice") or only the acetone vehicle ("control mice"). A second challenge was similarly delivered 24 h later.

2.4. Behavioral testing

All behaviors were assessed 24 h after the second challenge of SADBE or acetone. Each of two mice was placed in a separate, clear, plastic container, each 9 × 9 × 13 cm. A small amount of bedding was placed in each container to absorb any urine voided by the mouse. A camcorder (Panasonic HDC-HS250 high definition video camera) was positioned above the mice to record the behavior of the two mice at the same time. There were four angled mirrors, one on each side of each container, affording the camera a four-sided view in addition to the view from the top. Experiments were conducted inside a sound proof room. Pseudo-white noise was delivered from a radio to mask extraneous laboratory noises. The experimenter was present briefly to start the video recording, and 30 min later, to inject a chemical stimulus.

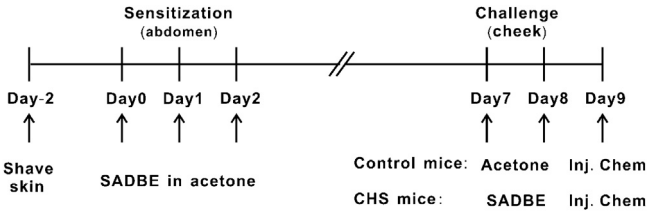

Fig. 1. The schematic experimental schedule for the induction of CHS. The mice were sensitized by the topical application of 25 μL of 1% SADBE in acetone to abdominal skin once daily for three consecutive days. Five days later, the right cheek was challenged either with a topical application of 25 μL of 1% SADBE in acetone ("CHS mice") or the acetone vehicle alone ("control mice") once a day for 2 consecutive days. Twenty-four hours later after the second challenge, a pruritic or algesic chemical was intradermally injected into the right cheek.

2.5. Experimental protocol

At 24 h after the second challenge, each mouse was placed in the test container and its spontaneous behavior was recorded for 30 min. Then each mouse received an intradermal injection into the previously challenged cheek of 5 μL of a chemical solution (via a 0.3 mL insulin syringe with a 31 gauge needle) and returned to the container to have its behavior recorded for another 30 min. For different groups of 8 mice each, the solution consisted of a normal saline vehicle alone or the vehicle containing either histamine (5 μg), BAM8-22 (1 μg) or bradykinin (2.65 μg).

2.6. Behavioral analyses

The video recording was played back on a Blu-ray player connected to a HDTV screen. The number of bouts of scratching and the number of wipes directed to the mouse cheek were scored in bins of one minute. A scratching bout was defined as one or more rapid back-and-forth motions of the ipsilateral hind paw directed toward the injected cheek, and ending with placement of the hind paw on the floor and/or to the mouth. A wiping was defined as a motion of the ipsilateral forelimb beginning at the back of the cheek, and moving forward in a caudal to rostral direction [3]. Wiping and scratching behaviors were included only if directed to the site of the intradermal injection on the cheek. Simultaneous wiping with both forelimbs (grooming) or a unilateral wiping or scratching directed to loci other than the cheek such as the bridge of the nose, the eye, ear, snout, or neck were identified and then excluded.

2.7. Statistical analysis

The significance of differences in the mean numbers of a particular behavior obtained for two different experimental conditions was tested using Student's *t*-test. The criterion for significance was $P < 0.05$. In cases where the assumption of an equality of variances was unmet as determined by Levene's test, the nonparametric Mann–Whitney *U* test was used instead. The data in bar graphs in the figures are the means and standard errors of the mean (SEM).

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