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BEHAVIORAL MODEL OF ITCH, ALLOKNESIS, PAIN AND ALLODYNIA IN THE LOWER HINDLIMB AND CORRELATIVE RESPONSES OF LUMBAR DORSAL HORN NEURONS IN THE MOUSE

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Abstract—We have further developed a behavioral model of itch and pain in the lower hindlimb (calf) originally reported by LaMotte et al. (2011) that allows comparisons with responses of lumbar dorsal horn neurons to pruritic and noxious stimuli. Intradermal (id) microinjection of the pruritogens histamine, SLIGRL-NH2 (agonist of PAR-2 and MrgprC11) and chloroquine (agonist of MrgprA3) into the calf of the lower limb elicited significant biting and a small amount of licking directed to the injection site, over a 30-min time course. Following id injection of histamine, low-threshold mechanical stimuli reliably elicited discrete episodes of biting (alloknesis) over a longer time course; significantly less alloknesis was observed following id injection of SLIGRL-NH2. Capsaicin injections elicited licking but little biting. Following id injection of capsaicin, low-threshold mechanical stimuli elicited discrete hindlimb flinches (allodynia) over a prolonged (>2 h) time course. In single-unit recordings from superficial lumbar dorsal horn neurons, low-threshold mechanically evoked responses were significantly enhanced, accompanied by receptive field expansion, following id injection of histamine in histamine-responsive neurons. This was not observed in histamine-insensitive neurons, or following id injection of saline or SLIGRL-NH2, regardless of whether the latter activated the neuron or not. These results suggest that itch-responsive neurons are selectively sensitized by histamine but not SLIGRL-NH2 to account for alloknesis. The presently described ''calf'' model appears to distinguish between itch- and pain-related behavioral responses, and provides a basis to investigate lumbar spinal neural mechanisms underlying itch, alloknesis, pain and allodynia. © **2014 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: itch, alloknesis, pain, allodynia, scratching, dorsal horn neuron.

INTRODUCTION

Chronic itch frequently accompanies many dermatological and systemic diseases (Ikoma et al., 2006). The associated scratching exacerbates the skin inflammation, leading to a vicious itch-scratch cycle (Wahlgren, 1999) that reduces the quality of life (Hundley et al., 2006). A potential mechanism is sensitization of itch-signaling pathways, manifested by spontaneous itch and scratching, hyperknesis (enhanced itch), and alloknesis (touch-evoked itch) (Hosogi et al., 2006; Ikoma et al., 2006; for recent review, see Akiyama and Carstens, 2013). Itch can be elicited by innocuous touching of normal skin around a site of histamine-evoked itch (Bickford, 1937; Graham et al., 1951; Simone et al., 1991; Heyer et al., 1997). In chronic itch patients low-threshold mechanical stimuli, such as clothes contacting the skin, can initiate the itch-scratch cycle (Bendsoe et al., 1987; Wahlgren, 1991; Heyer and Hornstein, 1999; Ricci et al., 2006; Mason, 2008).

To develop mechanism-based treatments for itch, animal models are required. Scratching behavior in rodents is commonly used to assess itch (Carstens and Kuraishi, 2004). This traditionally involves counting hindlimb scratch bouts directed toward a pruritogen injection in rostral back skin. A drawback is that hindlimb scratching does not discriminate between itch and pain since it is the only available response. We developed a novel model of alloknesis in which touch near a site of intradermal (id) injection of histamine and certain other pruritogens, or at the edge of an area of dry skin, reliably elicited hindlimb scratches (Akiyama et al., 2012a). In a recent variant, id pruritogen injection in the rodent cheek elicited primarily hindlimb scratching, while algogens elicited mainly forelimb wiping (Shimada and LaMotte, 2008). Scratching was reduced by systemic administration of μ-opioid antagonists whereas wiping was reduced by systemic administration of morphine (Akiyama et al., 2010a; Spradley et al., 2012b). Furthermore, this model allows direct comparisons of behavior with pruritogen- and algogen-evoked responses of first- and second-order trigeminal neurons (Akiyama et al., 2010b; Klein et al., 2011a). For decades, however, much information about itch and pain has come from recordings of lumbar spinal neurons with input from the hindlimb. Moreover, it is important to recognize differences in trigeminal vs. spinal processing of itch and pain (e.g., Spradley et al., 2012b). Thus, a behavioral model that distinguishes between itch and pain from the lower hindlimb is desirable.

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Abbreviations: id, intradermal; WDR, wide dynamic range; HT, high-threshold; 5-HT, 5-hydroxytryptamine.

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Hindpaw injection of 5-hydroxytryptamine (5-HT) elicited naloxone-sensitive paw-biting accompanied by licking, while hindpaw injection of algogens only elicited paw-licking (Hagiwara et al., 1999). Mice with chronic dry hindlimb skin exhibited spontaneous paw-biting (Nojima et al., 2004) that was sensitive to naltrexone but not morphine (Akiyama et al., 2010c). Lumbar dorsal horn neurons ipsilateral to the dry skin-treated hindpaw exhibited elevated spontaneous activity (Akiyama et al., 2011a,b). A minor drawback of the hindpaw behavioral model is that weight-bearing and locomotion may interfere with itch-related behaviors. It was recently reported that id injection of histamine into the mouse calf elicited biting whereas injection of capsaicin elicited licking (LaMotte et al., 2011). In the present study, we have further developed and validated this calf model. We additionally developed a novel model of alloknesis and allodynia, whereby low-threshold mechanical stimuli reliably elicit biting or hindlimb flinches, respectively, following id injection of histamine or capsaicin. Finally, in electrophysiological experiments we tested whether id injection of histamine enhances the mechanosensitivity of pruritogen-responsive lumbar superficial dorsal horn as a possible mechanism underlying alloknesis.

EXPERIMENTAL PROCEDURES

Experiments were conducted using adult C57BL/6 mice (Harlan, Oxnard CA, USA) (19–32 g body weight) under a protocol approved by the UC Davis Animal Care and Use Committee.

Behavior

The fur on the calf was shaved and mice were habituated to a Plexiglas recording arena with a transparent cover one week prior to testing. On the test day, the animal was restrained by hand and the skin on one hind limb was mildly stretched. An id microinjection of 10 μl was made in the calf of one of the following: vehicle (isotonic saline), histamine (50 μg; Sigma–Aldrich, St. Louis, MO, USA), SLIGRL-NH2 (50 μg; Quality Controlled Biochemicals, Hopkinton, MA, USA), chloroquine (50 μg; Sigma), capsaicin (10 μg; Sigma), or 7% Tween-80 (vehicle for capsaicin). The calf id microinjection was made using a 30-G needle attached to a Hamilton microsyringe by PE-50 tubing. Immediately following the id microinjection, mice were placed into a clear glass arena containing mirrors set at angles to allow multiple views of the animal that were captured with a high-definition videocamera. Investigators left the room during videotaping. The videocamera was set at high definition and slow motion capture modes to facilitate the assessment of biting and licking behaviors directed to the injected calf in a frame-by-frame video playback conducted offline by two investigators blinded as to the experimental treatment. The duration of biting, licking and flinching episodes was timed at 5-min intervals over the 50-min recording period. A bite is defined as direct contact of the incisors with calf skin. Biting was accompanied by relatively high-frequency low-excursion head movements which were used as an adjunct measure. The duration of each biting action was timed with an original program which allows us to check the movie frame by frame, and individual bite durations were summed to provide the cumulative biting time. The cumulative duration of licking, defined as direct contact of the calf skin by tongue protrusion, was also measured. Licks were accompanied by head movements that were of lower frequency and larger excursion compared to those associated with biting, and were also used as an adjunct measure of licking.

In a separate experiment, the animal was habituated to a recording arena for 1 h and then tested for alloknesis and allodynia at 5-min intervals, starting 5 min post-histamine or capsaicin injection. Alloknesis and allodynia were assessed as follows. At 5-min intervals, the mouse received three separate innocuous mechanical stimuli delivered using a von Frey filament (bending force: 0.7 mN). Stimuli were delivered 2 mm or further distal to the edge of the visible bleb formed by the id injection of histamine or other agents. The 0.7 mN von Frey filament was selected because it never elicited behaviors (i.e. biting, licking and flinching) in naïve mice, and was the minimum strength to elicit biting when delivered to skin surrounding the site of histamine injection. The mice were videotaped with a high-speed recording feature that turns 3 s of video into 12 s of slow motion video. The presence or absence of a positive response, i.e., biting, licking and flinching directed to the site of mechanical stimulation, was confirmed by video playback. The behavior score was the total number of positive responses elicited by the three stimuli, i.e., 0, 1, 2 or 3. The sequence was repeated at 5-min intervals out to 60 min post-injection, and again at 90 and 120 min post-injection time points. In experiments with histamine and capsaicin, an overall behavior score per 60 min and 120 min, respectively, was calculated as the sum of individual behavior scores.

Electrophysiological recording

The mouse was anesthetized with sodium pentobarbital (60 mg/kg i.p.) and prepared for single-unit recording from the lumbar spinal cord as previously detailed (Akiyama et al., 2009b). A tungsten microelectrode was driven into the superficial lumbar dorsal horn and single extracellularly recorded units were isolated using mechanical touch and pressure stimuli delivered to the ipsilateral hindpaw. In a few experiments we recorded from units that responded to mechanical stimulation of the ipsilateral calf. However, we decided to focus on units with hindpaw receptive fields because data from such units may be compared with data from our previous electrophysiological studies (Akiyama et al., 2009a,b, 2011a,b), and it was more difficult to accurately map receptive field dynamics on the calf compared to hindpaw. Recording depths were restricted to <300 μm below the surface as in our previous study. Unit activity was amplified, digitized and displayed on computer using a Powerlab (AD Instruments, Colorado Springs, CO, USA) interface. Once a mechanosensitive unit was isolated, we tested the unit responsiveness to