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SCRATCHING INHIBITS SEROTONIN-EVOKED RESPONSES OF RAT DORSAL HORN NEURONS IN A SITE- AND STATE-DEPENDENT MANNER

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Abstract—Scratching inhibits pruritogen-evoked responses of neurons in the superficial dorsal horn, implicating a spinal site for scratch inhibition of itch. We investigated if scratching differentially affects neurons depending on whether they are activated by itchy vs. painful stimuli, and if the degree of inhibition depends on the relative location of scratching. We recorded from rat lumbar dorsal horn neurons responsive to intradermal (id) microinjection of serotonin (5-hydroxytryptamine, 5-HT). During the response to 5-HT, scratch stimuli (3 mm, 300 mN, 2 Hz, 20 s) were delivered at the injection site within the mechanosensitive receptive field (on-site), or 4–30 mm away, outside of the receptive field (off-site). During off-site scratching, 5-HT-evoked firing was significantly attenuated followed by recovery. On-site scratching excited neurons, followed by a significant post-scratch decrease in 5-HT-evoked firing. Most neurons additionally responded to mustard oil (allyl isothiocyanate). Off-site scratching had no effect, while on-site scratching excited the neurons. These results indicate that scratching exerts a state-dependent inhibitory effect on responses of spinal neurons to pruritic but not algesic stimuli. Moreover, on-site scratching first excited neurons followed by inhibition, while off-site scratching immediately evoked the inhibition of pruritogen-evoked activity. This accounts for the suppression of itch by scratching at a distance from the site of the itchy stimulus.

Key words: rat, serotonin, scratch, itch, dorsal horn neuron, inhibition.

INTRODUCTION

Itch is often defined as an unpleasant sensation associated with the desire to scratch. Itch sensation presumably provides a warning signal that an organism or plant spicule has invaded the skin surface, and triggers scratching to remove the offending stimulus. Scratching and a variety of other noxious counterstimuli relieve itch sensation in humans (Murray and Weaver, 1975; Ward et al., 1996; Yosipovitch et al., 2005). The mechanism by which scratching the skin surface relieves itch is thought to involve the inhibition of spinal itch-signaling neurons (Davidson et al., 2009; Akiyama et al., 2011, 2012).

We have presently investigated the effect of scratching on presumptive itch-signaling neurons in the rat spinal cord. Sprague–Dawley rats exhibit pain-like responses to the prototypical itch mediator, histamine (Carstens, 1997; Klein et al., 2011), but exhibit itch-related hindlimb scratching behavior following an intradermal (id) injection of serotonin (5-hydroxytryptamine, 5-HT) (Berendsen and Broekkamp, 1991; Thomsen et al., 2001; Jinks and Carstens, 2002). 5-HT-evoked scratching involves 5-HT2 receptors (Nojima and Carstens, 2003a) and is inhibited by μ-opioid antagonists but not agonists (Nojima et al., 2003; Nojima and Carstens, 2003b; Spradley et al., 2012). A subpopulation of rat dorsal root ganglion (DRG) neurons with primary afferent C-fibers gave prolonged responses to peripheral cutaneous application of 5-HT (Hachisuka et al., 2010). Superficial dorsal horn neurons in the rat lumbar spinal cord respond to id injection of 5-HT (Carstens, 1997; Jinks and Carstens, 2002; Jinks et al., 2002; Nojima et al., 2003) over a time course matching that of 5-HT-evoked scratching (Jinks and Carstens, 2002). 5-HT-responsive neurons also responded to a variety of algogens (Carstens, 1997; Jinks and Carstens, 2002). Substance P participates as a spinal neuropeptide transmitter in 5-HT-evoked scratching, which was significantly attenuated following neurotoxic destruction of neurons in the superficial medullary and cervical dorsal horn that express neurokinin-1 (NK-1) receptors (Carstens et al., 2010). Gastrin-releasing peptide is another neuropeptide implicated in the spinal transmission of itch (Sun and Chen, 2007; Sun et al., 2009). Thus, using 5-HT as a pruritogen, the Sprague–Dawley rat presents an attractive animal model to investigate spinal mechanisms of itch and its modulation. In the present study, we have investigated the effect of scratching on responses of presumptive itch-signaling spinal neurons to the cutaneous application of 5-HT and the algogen, allyl isothiocyanate (AITC; mustard oil) which elicits a burning pain sensation in humans.

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Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); AITC, allyl isothiocyanate; ANOVA, analysis of variance; DRG, dorsal root ganglion; Mrgpr, mas-related G-protein-coupled receptor; NK-1, neurokinin-1; NS, nociceptive-specific; PSTH, peristimulus-time histogram; TRPV1, transient receptor potential vanilloid-1; VGLUT-2, vesicular glutamate transporter-2; WDR, wide dynamic range.

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EXPERIMENTAL PROCEDURES

Experiments were performed using 13 adult male Sprague–Dawley rats (Simonsen, Gilroy, CA, USA; 420–660 g) under a protocol approved by the UC Davis Animal Care and Use Committee. The single-unit recording from the lumbar spinal cord was conducted as previously detailed (Jinks and Carstens, 2002). Anesthesia was induced by sodium pentobarbital (60 mg/kg ip) and maintained by intravenous infusion of pentobarbital (10–20 mg/kg/h). A tungsten microelectrode recorded single-unit activity in the lumbosacral spinal cord. A chemical search strategy (Jinks and Carstens, 2002) identified and isolated 5-HT-responsive units. Briefly, a small (~0.1 μl) intradermal (id) microinjection of 5-HT (1%; 47 mM; Sigma–Aldrich, St. Louis, MO, USA) was made in the ventral hindpaw and a unit in the superficial lumbar dorsal horn (depth < 300 μm) exhibiting ongoing activity was isolated. After the ongoing activity subsided, 1 μl of 5-HT was injected through the same needle. Only units exhibiting an increase of > 30% in firing were selected for further study.

During a period of relatively stable elevated firing following id 5-HT, a series of scratch stimuli was delivered either directly at the injection site (on-site), or at a site 4–30 mm away (off-site). Scratch stimuli consisted of back-and-forth movements of a brush bristle resembling a rat claw across the hindpaw skin at a constant frequency of 2 Hz, excursion of 3 mm, force of 300 mN, and duration of 20 s. The scratch stimulus was applied either on- or off-site, in randomized order with at least 60 s between scratching at either site.

5-HT-evoked activity usually decreased toward pre-injection levels after 1 h. In most cases we were able to test the effect of scratching on neuronal activity following id injection of vehicle (id saline, $n = 6$) and topical (2 μl) application of 75% AITC ($n = 9$) or mineral oil ($n = 6$). Ten of 12 5-HT-responsive neurons responded to AITC, similar to our previous report (Jinks and Carstens, 2002).

After testing effects of scratching on evoked responses, the neurons were tested for mechanosensitivity. Using a von Frey filament (bending force, 55 mN), we determined the border between locations at which the mechanical stimulus either did, or did not, elicit a reproducible response to at least three of five stimulus applications. This border was taken as the perimeter of the receptive field. As noted above, on-site scratching was delivered at the injection site which was always located within the receptive field, while off-site scratching was delivered at distances of 4–30 mm from the injection site which was always outside the perimeter of the receptive field. Units were classified as wide dynamic range (WDR) if they gave graded responses to progressively stronger stimuli, or nociceptive-specific (NS) if they responded to noxious pinch but not to low-threshold von Frey, cotton wisp or brush stimuli.

Action potentials were recorded on computers running Spike2 (CED, Cambridge, UK) and Chart software (AD Instruments, Colorado Springs, CO, USA) and usually quantified as spikes/s. Mean firing rate was calculated over 1-min periods before and after the injection of 5-HT, AITC or vehicles. Ongoing responses were summed over 20-s intervals before, during and after on- or off-site scratching, and compared by the use of repeated-measures analysis of variance (ANOVA) followed by a post hoc Bonferroni test, with $P < 0.05$ set as significant. At the conclusion, an electrolytic lesion was made at the spinal cord recording site. The spinal cord was postfixed in 10% buffered formalin, cut in 50-μm frozen sections, and examined under the light microscope to identify lesions.

RESULTS

Data were collected from 13 5-HT-responsive neurons located in the superficial dorsal horn (Fig. 3E) at a mean depth of 146.4 ± 33.7 (SE) μm from the surface. All units responded to scratching. Twenty percent responded at lower frequency to low-threshold mechanical stimuli and were classified as wide dynamic range (WDR), while the remainder were classified as nociceptive-specific (NS).

5-HT increased firing to a level that was significantly higher compared to the pre-injection baseline or to vehicle- (saline-) evoked firing (Fig. 1). Ten of 12 (83%) units tested responded to AITC, which elicited a significant increase in firing compared to the pre-injection baseline and to vehicle (mineral oil) application (Fig. 1). An example is shown in Fig. 2. This unit was located in the superficial dorsal horn and responded to id 5-HT. During the 5-HT-evoked response, on-site scratching further excited the neuron, while off-site scratching inhibited ongoing activity (Fig. 1, left-hand peristimulus-time histogram (PSTH)). Saline did not elicit a response. Following id saline, on-site scratching also excited the unit while off-site scratching had no effect (Fig. 2, middle PSTH). The unit responded to a second id injection of 5-HT (Fig. 2, 3rd PSTH from left). Application of mineral oil (AITC vehicle) had no effect,

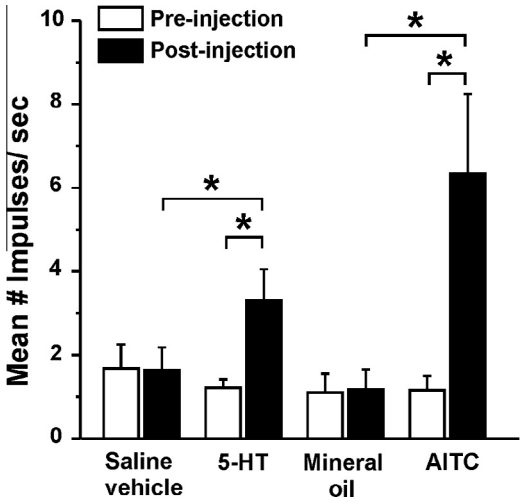

Fig. 1. Activation of superficial dorsal horn neurons by 5-HT, AITC and vehicles. Bar graph plots mean firing rate (calculated over 60 s) pre- (open bars) and post-injection (filled bars). Error bars: SEM. *Significantly different ($p < 0.05$, paired t-test).