Itch-Associated Responses of Afferent Nerve Innervating the Murine Skin: Different Effects of Histamine and Serotonin in ICR and ddY Mice

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ABSTRACT—To assess the itch-associated response of primary afferents innervating the murine skin in vivo, dose-response curves and time-courses for itch-scratching and cutaneous nerve firing responses to intradermal injections of pruritogens (histamine and serotonin) were compared in ICR and ddY mice. Serotonin increased the itch-scratch response and cutaneous nerve firing in either ICR or ddY mice. Histamine increased these two responses in ICR, but not ddY, mice. The dose-response curves and time-courses for serotonin- and histamine-induced nerve firing were similar for the two strains. The results suggest that cutaneous nerve firing evoked by peripherally given pruritogens includes the itch-associated response.

Keywords: Itch-scratch response, Cutaneous nerve firing, Strain difference

Pruritus is a common symptom of many inflammatory cutaneous disorders (e.g., eczema, psoriasis, atopic dermatitis) (1). A major problem in the study of itch results from its subjective nature, which makes it difficult to quantify the degree. Therefore, clinicians have devised indirect methods, especially for the measurement of scratching, to obtain objective and quantifiable data (2). For objective evaluation of itch in animals, scratching by hind paws is also observed (3 – 6). Although the scratching is convenient to quantify, the reaction is composed of an itch reflex and behavioral response to an itch sensation. Accordingly, the itch-scratching response is easily influenced by locomotor activity and a mental state and not fully suitable to estimate the degree of cutaneous itch stimulation.

Pruritus is produced primarily near the dermal-epidermal junction (7), and then the itch signal is conveyed through the excitation of primary afferents to the dorsal horn. In that sense, it is important to measure the activity of cutaneous nerve evoked by application of known pruritogens. Although recording of the activity of afferent nerve innervating the skin is indispensable for the study of itch, such a method has not yet been established in animals. Therefore, we attempted to record the itch-associated response of afferent nerve innervating the skin of mice in vivo. Histamine and serotonin, which are inflammatory chemical mediators, can act as pruritogens not only in human subjects but also in mice (3, 4, 8). However, there is an obvious strain difference in the itch-scratch response to histamine between ICR and ddY mice (3, 6) and there is some evidence against the role of histamine as a common itch mediator in mice (5, 9). Hence, we compared the effects of histamine and serotonin on the nerve activity with the effects on the itch-scratch response in these strains of mice.

Male mice (ICR or ddY strain, 5- to 6-week-old) were used. Mice were housed under controlled temperature (23 – 25°C) and light (lights on from 08:00 to 20:00). Food and water were freely available. Before the behavioral experiment, each mouse was put into a clear plastic cage for about 60 min for acclimation. Immediately after intradermal (i.d.) injection of serotonin and histamine, dissolved in saline, in a volume of 50 μl into the rostral part of the back, they were put back into the same cage for the observation of scratching of the injection site by a hind paw. The behaviors were videotaped and the tapes were played back to count the itch-scratch response. The number of response was counted every 3 min for 60 min after injection. In the electrophysiological experiment, mice were anesthetized with an intraperitoneal injection of urethane (1.5 g/kg). The animals were lying on a heating board (37°C). The skin of the rostral back (about 1.5 cm in mediolateral width and about 2.5 cm in rostrocaudal length) was turned inside out. The cutaneous nerve branch was then exposed, dissected free from surrounding tissue and maintained in a pool of mineral oil. The nerve action potentials were
recorded extracellularly using bipolar electrodes of silver wire and an AC bioelectric amplifier (AB651; Nihon Kohden, Tokyo) with a band-pass filter of 1 kHz. The data were stored in a DAT data recorder (RD-135T; TEAC, Tokyo). The number of action potentials with amplitude over the action potentials synchronizing with breathing exercise and heart beat were estimated every 3 min, using a data analyzing system with software to produce a spike-height histogram (PowerLab/8s; AD Instruments Pty, Castle Hill, Australia). A solution of pruritogen (50 µl) was injected i.d. into the receptive region after a stable nerve activity was obtained.

The time courses of cutaneous nerve firing and itch-scratch responses following serotonin (Sigma, St. Louis, MO, USA) and histamine (Wako Pure Chemical Industries, Osaka) at a dose of 100 nmol/site were compared in ICR and ddY mice. In ICR mice, cutaneous nerve firing and itch-scratch response appeared within a few min after serotonin injection; peaked at 6–9 min and 6–15 min, respectively; and almost subsided within 60 min (Fig. 1: A and B). Both histamine-induced nerve firing and itch-scratch response came out within a few min, peaked at 3–9 min, and almost subsided by 30 min (Fig. 1C). In ddY mice, serotonin also induced the itch-scratch response and cutaneous nerve firing (Fig. 1D), the time courses of these responses being similar to those in ICR mice. On the other hand, histamine elicited neither the itch-scratch response nor cutaneous nerve firing (Fig. 1E).

To determine whether cutaneous nerve firing evoked by serotonin and histamine in ICR mice would result from activation of the respective receptors, the effects of the receptor antagonists were examined. We first examined the
Fig. 2. Effects of cyproheptadine (CYP) and chlorpheniramine (CHP) on the serotonin (5-HT)- and histamine-induced cutaneous nerve firing in anesthetized ICR mice. A, B Effect of 5-HT on nerve activity (A) and its inhibition by CYP (B). C, D Effect of histamine on nerve activity (C) and its inhibition by CHP (D). 5-HT (100 nmol/site, ●), histamine (100 nmol/site, ○) and saline (SAL, □) were administered intradermally into the receptive region. CYP (0.1 mg/kg) and CHP (3 mg/kg) were administered subcutaneously into the caudal back 15 min before 5-HT or histamine injection. The data of nerve activity elicited by 5-HT and histamine were from Fig. 1. The number of events per 3 min were plotted against time. Values are means ± S.E.M. of 4–10 animals.

Effects of two antagonists, the serotonin receptor antagonist cyproheptadine (Sigma) and the H1 histamine receptor antagonist chlorpheniramine (Research Biochemicals International, Natick, MA, USA) on serotonin- and histamine-induced scratches, respectively. When applied subcutaneously 15 min before the pruritogens (i.d., 100 nmol/site), cyproheptadine at a dose of 0.1 mg/kg decreased serotonin-induced scratches from 198 ± 44 (mean ± S.E.M., n = 8) to 77 ± 6 (n = 8) and chlorpheniramine at a dose of 3 mg/kg decreased histamine-induced scratches from 93 ± 18 (mean ± S.E.M., n = 8) to 34 ± 5 (n = 8). We next examined the effects of the antagonists on the nerve activity. An i.d. injection of saline into the receptive field did not evoke the nerve firing (Fig. 2A). Serotonin (100 nmol/site)-induced nerve firing was abolished by systemic pretreatment with cyproheptadine (0.1 mg/kg, subcutaneously 15 min before serotonin) (Fig. 2: A and B). Histamine (100 nmol/site)-induced nerve firing was abolished by systemic pretreatment with chlorpheniramine (3 mg/kg, subcutaneously 15 min before histamine) (Fig. 2: C and D), suggesting the involvement of H1 histamine receptors. In our preliminary experiments, when injected i.d. together with histamine or serotonin, chlorpheniramine inhibited scratching induced by histamine but not by serotonin, suggesting that histamine is not involved in the scratch-inducing action of serotonin. Therefore, cyproheptadine might inhibit the serotonin effect on nerve activity chiefly through the blockade of 5-HT1 or 5-HT2 serotonin receptors, although this antagonist blocks H1 histamine receptors as well as 5-HT1/2 serotonin receptors. Since multiple subtypes of serotonin receptors and H1 receptors are located on primary afferents (10, 11), the evoked nerve firing may be at least partly produced via activation of the respective receptors on primary afferents.

In either the ICR or ddY strain, serotonin dose-dependently increased itch-scratch responses at intradermal doses up to 100 nmol/site and the higher doses rather inhibited the itch-scratch response (Fig. 3: A and B). The cutaneous nerve activity was also increased by serotonin at doses up to 100 nmol/site and rather decreased by the higher doses (Fig. 3: A and B). In ICR mice, histamine dose-dependently increased itch-scratch responses at intradermal doses up to 1,000 nmol/site, the dose-response curve being gentler than that for serotonin (Fig. 3A). The effect of histamine on cutaneous nerve activity reached a low ceiling at doses of 100–300 nmol/site (Fig. 3A). In ddY mice, histamine did not significantly increase itch-scratch response and cutaneous nerve activity (Fig. 3B).

One important finding in the present experiments is that itch-scratch response and cutaneous nerve firing following pruritogen injection were similar to each other in the time-course and dose-response relationship. It was shown that serotonin and histamine increase the cutaneous nerve firing in cats, in which these amines were used as pain-producing substances (12). However, in mice, cutaneous injection of serotonin elicits itch-related rather than pain-related behavioral responses (6, 13), suggesting that serotonin is pruritogenic rather than algogenic in mice. With regard to hista-
serotonergic receptors to suppress the serotonin-induced activation of cutaneous nerve. Which subtype of serotonin receptors are involved in this suppression is an interesting question to be elucidated in future studies. Serotonin elicits pain when administered to the cantharidin blister area in human subjects and painful stimulation is known to inhibit an itch sensation. Thus, another explanation for the bell-shaped dose-response curve for serotonin-induced scratching is that higher doses of serotonin elicit pain to inhibit itch signaling. However, high doses of serotonin did not increase the firing of cutaneous nerve. In addition, an injection of high doses of serotonin into the hind paw did not elicit the pain-associated response in mice (13). Therefore, this may not be the primary cause of the inhibition of the itch-scratch response. We can not deny the possibility that high doses of serotonin released inhibitory factors from the skin, which resulted in the inhibition of the serotonin action. Thus, the mechanisms of inhibition by higher doses of serotonin remain obscure.

Histamine increased the firing of cutaneous nerves as well as the itch-scratch response in ICR mice, but not in ddY mice. The simplest explanation of this strain difference is that H1 histamine receptors are expressed on itch-signaling sensory neurons in ICR mice but not in ddY mice. However, in our preliminary experiments, the expression level of H1 receptor mRNA in the dorsal root ganglia of ddY mouse was similar to that of ICR mouse. Another explanation is that histamine induced plasma extravasation in ICR mice but not in ddY mice and that the exudate induced itch-associated responses. However, an i.d. injection of histamine to the rostral back elicits apparent plasma extravasation in ddY mice as well as ICR mice (15). Therefore, there may not be any apparent strain difference in histamine action on endothelial cells, and plasma exudate may not play an important role in the pruritic action of histamine in ICR mice. Thus, further experiments are needed to elucidate the cause of the strain differences.

In summary, we found that the dose-response curves and time-courses for cutaneous nerve responses to pruritogens were similar to those for the itch-scratch responses. The results suggest that cutaneous nerve firing evoked by a peripherally given pruritogen at least partly includes the itch-associated response. Thus, the recording of the activity of cutaneous nerves and their response to pruritogen may be useful for the study of mechanisms of production and conduction of itch signals in the periphery and peripheral mechanisms of anti-pruritogenic drugs.

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