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Short Communication

Activation of Proteinase-Activated Receptors Induces Itch-Associated Response Through Histamine-Dependent and -Independent Pathways in Mice

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Abstract. Proteinase-activated receptor-2 (PAR_2) participates in itch, but the role of the other subtypes of this receptor remain unknown. To investigate this issue, scratching, an itch-related behavior, was observed following intradermal injections of the activating peptides of $PAR_{1–4}$ in mice. Activating peptides of PAR_1, PAR_2, and PAR_4, but not PAR_3, induced scratching. The antihistamine terfenadine suppressed scratching elicited by activating peptides of PAR_1 and PAR_4, but not PAR_2. These results suggest that PAR_1, PAR_2, and PAR_4 are involved in itch and that histamine is a cause of itch related to PAR_1 and PAR_4, but not PAR_2.

Keywords: proteinase-activated receptor subtype, itch, scratch

Proteinases have long been known to elicit itch following administration to the human skin. Itch induced by some proteinases such as trypsin and chymase is though to be mediated by histamine released from mast cells, whereas itch induced by others such as kallikrein and papain may not be due to histamine release (1, 2). Recently, proteinase-activated receptor 2 (PAR_2) has been shown to be involved in itch. PAR_2-activating peptide (PAR_2-AP) provokes itch in the skin, especially atopic eczema, in humans (3) and it elicits scratching in mice (4). Tryptase, a PAR_2 agonist proteinase, induces scratching in mice, which is inhibited by anti-PAR_2 antibody and the PAR_2 antagonist FSLLRY-NH_2 (5). Scratching induced by compound 48/80, which degranulates mast cells, is mediated by histamine and tryptase (5). Different groups of primary sensory nerves may be involved in itch signals induced by histamine and tryptase (6).

PAR is a family member of G-protein–coupled receptors and the activation of PAR is initiated by cleavage of the N terminus of the receptor to generate a new tethered ligand terminus, which activates PAR itself (7). Synthetic peptides which have an amino acid sequence similar to the tethered ligand also activate PAR (7). Four PAR subtypes, $PAR_{1–4}$, have been identified. Tryptase acts on PAR_2 (and PAR_1 only at high concentration), trypsin on PAR_1, PAR_2, and PAR_4, but not PAR_3; and thrombin acts on PAR_1, PAR_3, and PAR_4, but not PAR_2 (7). As mentioned above, some evidence suggests the involvement of PAR_2 in itch, but the involvement of the other PARs remains unknown. Thus, the present study was conducted to compare the itch-inducing efficacy of selective agonists of $PAR_{1–4}$ and determine the involvement of histamine.

Male ICR mice (4 – 8 week-old), purchased from Japan SLC (Shizuoka), were used. They were housed in a room under controlled temperature (22 ± 2°C) and light (light on 07:00 – 19:00 h). Food and water were freely available. Procedures for animal experiments were approved by the Committee for Animal Experiments at the University of Toyama and were conducted in accordance with the animal experiment guidelines of The Japanese Pharmacological Society.

TFLLR-NH_2, SLIGRL-NH_2, SFNGGP-NH_2, and AYPGKF-NH_2, synthesized by JBL, were dissolved in saline and injected intradermally in a volume of 50 μl into the rostral skin, the hair of which was clipped the day before. Terfenadine (Sigma, St. Louis, MO, USA)

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was suspended in 0.5% sodium carboxymethylcellulose (Wako Pure Chemical Industries Ltd., Osaka) and was administrated orally 30 min before the agonist injection.

The mice were put into an acryl cage composed of four cells (13 × 9 × 35 cm) for at least 1 h for acclimation. Immediately after intradermal injection, they were put back into the same cells and the behaviors were videotaped for 1 h with personnel kept out of the observation room. Counts of scratching were made by video playback. The mice stretched either hind paw toward the injection site, leaned the head toward it, and rapidly scratched several times for about 1 s. A series of these movements was counted as one bout of scratching (8).

Data are presented as means and S.E.M. Statistical significance was analyzed using Dunnett's multiple comparisons; *P<0.05 was considered significant.

Intradermal injection of TFLLR-NH_2 (PAR_1-AP) at a dose of 100 nmol/site elicited scratching during the initial 10-min period and the lower doses of 10 and 30 nmol/site were without effects (Fig. 1). SLIGRL-NH_2 (PAR_2-AP) at a dose of 100 nmol/site elicited scratching during the initial 20-min period and dose-dependent effects were observed at doses of 10 – 100 nmol/site (Fig. 1). SFNGGP-NH_2 (PAR_3-AP) was without effects at doses of 10 – 100 nmol/site (Fig. 1). AYPGKF-NH_2 (PAR_4-AP) at a dose of 100 nmol/site elicited scratching during the initial 10-min period and the lower doses of 10 and 30 nmol/site were without effects (Fig. 1).

Scratch bouts induced by SLIGRL-NH_2 (100 nmol/site) was about twice those of TFLLR-NH_2 (100 nmol/site) and AYPGKF-NH_2 (100 nmol/site), and scratch bouts induced by SLIGRL-NH_2 (30 nmol/site) was less than that of AYPGKF-NH_2 (100 nmol/site). In this series of experiments, therefore, we tested the effects of the H_1 histamine–receptor antagonist terfenadine on the scratching induced by TFLLR-NH_2 (100 nmol/site), SLIGRL-NH_2 (50 nmol/site), and AYPGKF-NH_2 (100 nmol/site). Terfenadine (10 and 30 mg/kg) partially but significantly inhibited scratching induced by TFLLR-NH_2 and AYPGKF-NH_2, but the action of SLIGRL-NH_2 was not affected by terfenadine at the same doses (Fig. 2).

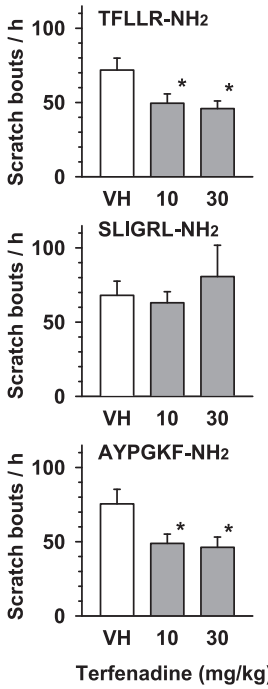

Fig. 2. Effects of the H_1 histamine–receptor angatonist terfenadine on scratching induced by PAR subtype-selective agonists in mice. Scratch bouts were counted for 1 h following the intradermal injections of TFLLR-NH_2, SLIGRL-NH_2, and AYPGKF-NH_2 (PAR_1-, PAR_2-, and PAR_4-selective agonists, respectively) at doses of 100, 50, and 100 nmol/site, respectively. Terfenadine and vehicle (VH) were administered orally 30 min before agonist injection. Values represent the means and S.E.M. for six to eight animals. *P<0.05, compared with VH (Dunnett's multiple comparisons).

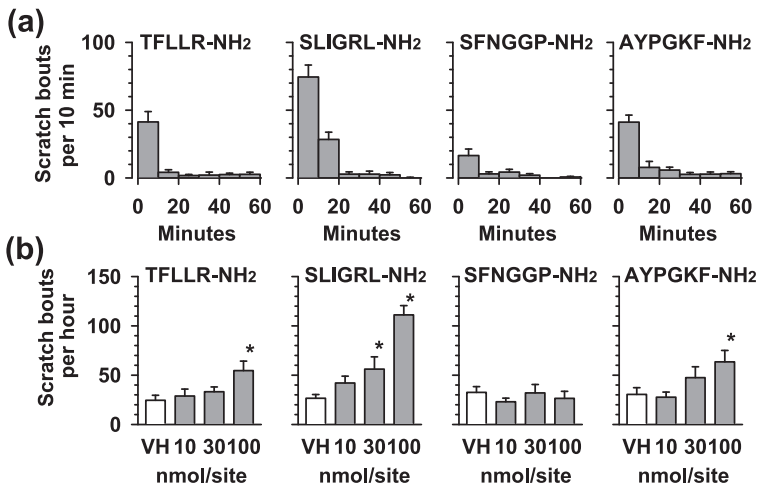

Fig. 1. Scratching following intradermal injections of PAR subtype-selective agonists in mice. a: Time-course of the effects of agonists (100 nmol/site). b: Dose-response effects for agonists. Scratch bouts were counted for 1 h following the intradermal injection of TFLLR-NH_2, SLIGRL-NH_2, SFNGGP-NH_2, AYPGKF-NH_2 (PAR_{1-4} agonists, respectively), or vehicle (VH, saline). Values represent the means and S.E.M. for six to seven animals. *P<0.05, when compared with VH (Dunnett's multiple comparisons).

TFLLR-NH_2 elicited an itch-associated response, which was partially inhibited by terfenadine. Considering that PAR_1 messenger RNA is expressed in the cutaneous mast cells of humans (9), TFLLR-NH_2 may act on mast cells to release histamine, which is involved in the effect of this agonist. PAR_1 messenger RNA is expressed in rat peritoneal mast cells, which do not release histamine after PAR_1 stimulation by thrombin and the PAR_1-activating peptide SFLLR-NH_2 (10). Thus, in contrast to cutaneous mast cells, peritoneal mast cells may not express functional PAR_1. Since the dorsal root ganglia express PAR_1 and TFLLR-NH_2 increases intracellular Ca^{2+} concentration in the dorsal root ganglion cells (11), direct activation of primary afferents by TFLLR-NH_2 may also be responsible for itch. PAR_1 is also expressed in keratinocytes (12), which produces several itch mediators including leukotriene B_4 (8). With these findings taken into account, the present results suggest that PAR_1 stimulation causes itch through histamine-dependent and independent mechanisms.

SLIGRL-NH_2 most potently induced scratching. Scratching induced by SLIGRL-NH_2 was not inhibited by terfenadine. The results are similar to the report of another group, in which scratching induced by SLIGRL-NH_2 is not inhibited by pyliramine, an H_1 histamine–receptor antagonist (4). Thus, functional PAR_2 receptors may not be expressed in the mouse cutaneous mast cells. Similarly, PAR_2 messenger RNA has been shown not to be present in the peritoneal mast cells of rats (10). In humans, the expression level of PAR_2 messenger RNA is relatively low, PAR_2 is expressed in about half of the cutaneous mast cells, and SLIGRL-NH_2 induces slight release of histamine form cultured cutaneous mast cells (9). Thus, the present results do not rule out the possibility that histamine is involved in itch after stimulation of PAR_2 in mast cells in humans. PAR_2 is expressed in several types of cells including keratinocytes and sensory neurons (3, 12). PAR_2 agonists including tryptase increase intracellular Ca^{2+} concentration in dorsal root ganglion neurons (13), raising the possibility that PAR_2 agonist activates directly primary afferents. PAR_2 is expressed in the keratinocytes, which may also play an important role in the induction of itch (8).

SFNGGP-NH_2 did not induce scratching. PAR_3 messenger RNA is expressed in the cutaneous mast cells of humans (9). Although thrombin acts on PAR_3 as well as PAR_4, it causes phosphoinositide hydrolysis in COS7 cells expressing PAR_4, but not PAR_3, and co-expression of PAR_3 and PAR_4 enhances the thrombin action, suggesting that PAR_3 itself does not mediate transmembrane signaling but instead functions as a cofactor for the activation of PAR_4 (14). Thus, PAR_3 alone may not be involved in the induction of itch, but the present results do not deny the possibility that it enhances PAR_4-mediated itching.

AYPGKF-NH_2 elicited itch-associated response, which was partially inhibited by terfenadine. PAR_4 is expressed in cutaneous mast cells of humans (9), but expressed in neither keratinocytes (12) nor primary afferents (15). Thus, the activation of PAR_4 in mast cells may release histamine that induces itch. Since the inhibition by terfenadine was only partial, there may be other mechanisms that remain unknown at present.

PAR_1, PAR_2, and PAR_4 were shown to be involved in itch. Trypsin-induced itch is suppressed by previous depletion of histamine at the injection site (1), which may be explained by the action of trypsin on PAR_1 and PAR_4 (7). Tryptase-induced scratching is markedly suppressed by anti-PAR_2 antibody (5). SLIGRL-NH_2–induced scratching was not inhibited by terfenadine. On the other hand, scratching induced by mast cell degranulation is partially suppressed by terfenadine and this inhibition is increased by nafamostat, a serine protease inhibitor (5), suggesting the involvement of both histamine and tryptase released from mast cells in itching. In humans, mast cell degranulation induces itch, which is inhibited by the H_1 histamine–receptor antagonist in healthy skin, but not in atopic eczema (3). The amount of tryptase released by mast cell degranulation is greater in atopic eczema than in healthy skin, although histamine release is not different between healthy skin and atopic eczema (3). Thus, the roles of PARs in itch may be different between healthy and pathologic conditions.

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