European Journal of Pharmacology 686 (2012) 16–21

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and Cellular Pharmacology

Cathepsin E induces itch-related response through the production of endothelin-1 in mice

Tsugunobu Andoh [a], Tetsuro Yoshida [a], Jung-Bum Lee [b], Yasushi Kuraishi [a,*]

[a] Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[b] Department of Pharmacognosy, Graduate School of Medical and Pharmaceutical Science, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

ARTICLE INFO

Article history:
Received 6 February 2012
Received in revised form 26 March 2012
Accepted 5 April 2012
Available online 21 April 2012

Keywords:
Cathepsin E
Endothelin-1
Itch
Keratinocyte
Mast cell
Scratching

ABSTRACT

This study investigated the pruritogenic potency of cathepsin E, an aspartic protease, and its mechanisms in mice. An intradermal injection of cathepsin E to the rostral back elicited scratching, an itch-associated response, of the injection site. This action was inhibited by the aspartic protease inhibitor pepstatin A, the endothelin ET_A receptor antagonist BQ-123, and the opioid receptor antagonists naltrexone and naloxone, but not by the H_1 histamine receptor antagonist terfenadine, the proteinase-activated receptor-2 antagonist FSLLRY-NH_2, or mast cell deficiency. Pepstatin A inhibited scratching induced by intradermal injection of the mast-cell degranulator compound 48/80, but not by tryptase, a mast-cell mediator. An intradermal injection of cathepsin E increased endothelin-1 levels in the skin at the injection site. Preproendothelin-1 mRNA was present in primary cultures of keratinocytes, and immunohistochemistry using an antibody recognizing endothelin-1 and big-endothelin-1 revealed immunoreactivity in the epidermis, especially in the prickle and granular cell layers, but not in the basal cell layer. These results suggest that cathepsin E is an endogenous itch inducer, and that its action is mediated at least in part by the production of endothelin-1 in the epidermis.

1. Introduction

Proteases have long been known to cause itching in humans; endopeptidases rather than exopeptidases cause itching (Arthur and Shelley, 1955), and mast cell-dependent and independent mechanisms are involved (Davies et al., 1979; Hägermark, 1973; Hägermark et al., 1972). Four proteinase-activated receptor subtypes (PARs) have recently been identified, including PAR_1, PAR_3, and PAR_4 receptors, which are thrombin receptors, and PAR_2, which is activated by a trypsin-type serine protease rather than thrombin (Macfarlane et al., 2001). Tryptase released from mast cells and PAR_2 receptors may be involved in itching in humans with atopic dermatitis (Steinhoff et al., 2003) and mice with atopy-like dermatitis (Tsujii et al., 2008, 2009). Since PAR_2 receptors are present in primary afferents and epidermal keratinocytes (Steinhoff et al., 2003; Tsujii et al., 2009), they may be important sites of pruritogenic activity of serine proteases. Cutaneous mast cells also contain chymase, a chymotrypsin-type serine protease (Metcalfe et al., 1997), and an intradermal injection of chymase causes itch in humans, probably by degranulating mast cells (Hägermark et al., 1972). Chymase is involved in the development of chronic dermatitis (Watanabe et al., 2002), but it is not pruritogenic in mice (Ui et al., 2006).

Cathepsin E (EC 3.4.23.34), an aspartic protease of the pepsin superfamily, is present in immune cells, such as lymphocytes and dendritic cells (for review see Zaidi and Kalbacher, 2008), epidermal keratinocytes (Hara et al., 1993), and mast cells (Henningsson et al., 2005). Cathepsin E plays a role in intracellular antigen processing in antigen-presenting cells (for review see Zaidi and Kalbacher, 2008) and in the processing of precursor peptides and proenzyme into mature peptides and active protease, respectively, in cells including mast cells (Henningsson et al., 2005; Kageyama et al., 1995). In addition, mast-cell degranulation results in the release of cathepsin E (Henningsson et al., 2005), which may process some precursor peptides in the extracellular space. In this context, cathepsin E converts big-endothelin-1 into mature peptide endothelin-1 (Lees et al., 1990). The pruritogenic activity of endothelin-1 is higher than those of other neuropeptides and histamine (Andoh et al., 1998, 2004; Maekawa et al., 2000; McQueen et al., 2007; Trentin et al., 2006). Thus, we investigated whether cathepsin E is pruritogenic in mice and, if so, which mechanism(s) are involved in its activity, including pruritogenic peptide (especially endothelin-1) production, histamine release, and PAR_2 receptor activation.

2. Materials and methods

2.1. Animals

Male ICR mice (5–8 weeks old) were used for all experiments except one series in which mast cell-deficient WBB6F1 W/W^v mice (8 weeks old) and their normal WBB6F1+/+ littermates were used. All mice were sourced from Japan SLC (Shizuoka, Japan). The mice were housed

* Corresponding author. Tel.: +81 76 434 7510; fax: +81 76 434 5045.
E-mail address: kuraisiy@pha.u-toyama.ac.jp (Y. Kuraishi).

0014-2999/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2012.04.024

in a room under controlled temperature (22±1 °C), humidity (55%±10%), and light (lights on from 07:00 to 19:00 h) conditions. Food and water were freely available. Procedures performed during the animal experiments were approved by the Committee for Animal Experiments at the University of Toyama.

2.2. Materials

Cathepsin E (R&D systems, Inc., Minneapolis, MN, USA) was dissolved in physiological saline and injected intradermally to the interscapular region or cheek in a volume of 50 or 10 μl, respectively. To inactivate cathepsin E, it was heated at 100 °C for 1 h. Endothelin-1 (Peptide Institutes, Inc., Osaka, Japan) and compound 48/80 (Sigma, St. Louis, MO, USA) were dissolved in physiological saline, and human tryptase (Wako Pure Chemical Ind., Osaka, Japan) was dissolved in phosphate-buffered saline (pH 7.4); they were injected intradermally as pruritogens. Naloxone hydrochloride and naltrexone hydrochloride were dissolved in physiological saline and injected subcutaneously 15 min before cathepsin E injection. Pepstatin A (Sigma) and BQ-123 (cyclo[D-Trp-D-Asp-L-Pro-D-Val-L-Leu]) (Peptide Institute) were injected together with pruritogens. Terfenadine was dissolved in 0.5% sodium carboxymethyl cellulose (Wako Pure Chemical Ind.) and administered orally 30 min before the cathepsin E and endothelin-1 injections.

2.3. Behavioral experiments

The day before the experiments, hair was removed from the rostral part of the back or cheek using hair clippers. Before behavioral observation, the animals were placed individually in an acrylic cage comprising 4 cells (13×9×35 cm) for at least 1 h to allow them to acclimate. Immediately after intradermal injection, the animals were placed back into the same cells and their behavior was videotaped for 1 h; all personnel were kept out of the observation room during this time. Play back of the video served for determination of hind-paw scratching (Kuraishi et al., 1995). Forelimb wiping of the face was used to evaluate pain-related response (Shimada and LaMotte, 2008).

2.4. Western blotting

Cathepsin E was injected into the rostral back of each mouse; 10 min later, the mice were euthanized by transcardiac perfusion of phosphate-buffered saline (PBS) under pentobarbital anesthesia (80 mg/kg, intraperitoneal). The skin of the injected region was excised, proteins were extracted from the skin samples with a lysis buffer (20 mM Tris HCl (pH 7.5), 137 mM NaCl, 1% NP-40, 10% glycerol, 1 mM phenylmethyl sulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin), and 20 μg of protein was separated by electrophoresis using a 10% sodium dodecyl sulfate-polyacrylamide gel then transferred to a polyvinylidene difluoride membrane. After blocking with 5% skim milk solution for 1 h, the membrane was reacted with rabbit anti-endothelin-1 antibody (Abbiotec) or mouse anti-β-actin antibody (Sigma) overnight at 4 °C. It was subsequently incubated with horseradish peroxidase-conjugated immunoglobulin G for 1 h at room temperature, and then treated with chemiluminescence reagents (Amersham Bioscience, Piscataway, NJ, USA). Chemiluminescent signals were detected using an X-ray film and analyzed with NIH Image software. The data was normalized to the level of β-actin.

2.5. Immunohistochemistry

The mice were transcardially perfused with PBS followed by 4% paraformaldehyde. The skin of the rostral back was removed, post-fixed for 4 h in the same fixative, and maintained for 1 week in PBS containing 30% sucrose at 4 °C. Frozen tissue sections were cut at 20 μm thickness using a cryostat. The sections were washed with PBS 3 times then incubated with PBS containing 0.3% Triton X-100 for 30 min. They were treated with 1% fetal bovine serum in PBS for 1 h and then with rabbit anti-endothelin-1 antibody (Abbiotec) or antibody treated with endothelin-1 (Peptide Institutes) and mouse anti-human keratin 14 monoclonal antibody for 24 h at 4 °C. After washing with PBS, the sections were incubated with Alexa Flour 488-conjugated anti-rabbit IgG antibody (Molecular Probes, Grand Island,

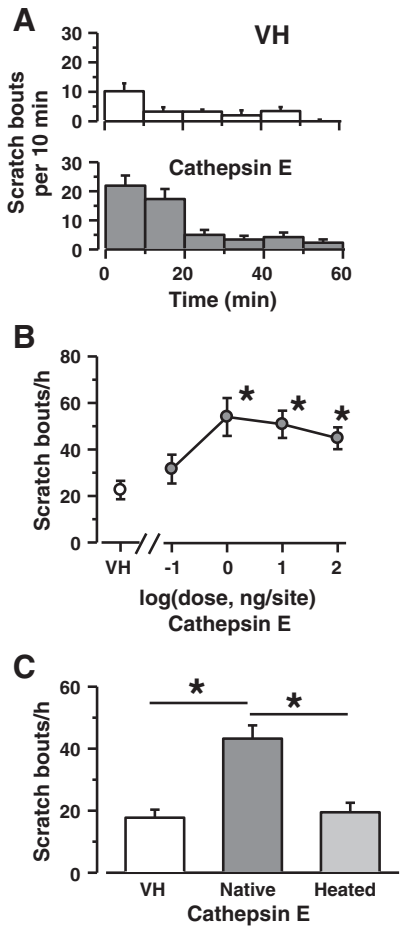

Fig. 1. Scratching responses to intradermal injections of cathepsin E into the rostral back of mice. Hind-paw scratching of the injection site was counted for 60 min after intradermal injection of cathepsin E and vehicle (VH). (A) Time-course of scratching after VH and cathepsin E (1 ng/site) injections. (B) Dose–response curve for cathepsin E. (C) Scratching after injection of cathepsin E (1 ng/site), heat-treated cathepsin E (1 ng/site), and VH. Values represent the means±S.E.M. for 11 to 12 animals. *$P<0.05$ (Bonferroni's test).

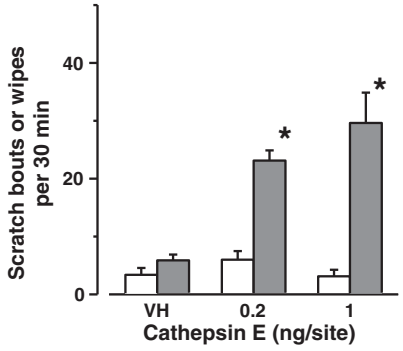

Fig. 2. Scratching and wiping responses to intradermal injections of cathepsin E into the cheek. Hind-paw scratching (shaded bars) and forelimb wiping (clear bars) of the injection site were counted for 30 min after the intradermal injection of cathepsin E (0.2 and 1 ng/site) and vehicle (VH) into the cheek. Values represent the means± S.E.M. for eight animals. *$P<0.05$ when compared with VH (Dunnett's test).

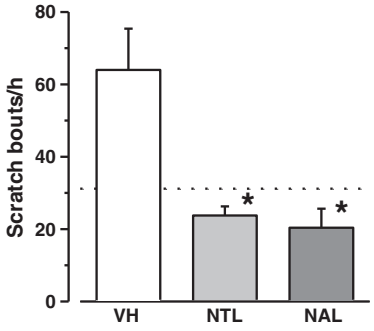

Fig. 3. Effects of opioid antagonists on scratching induced by cathepsin E injection into the rostral back. Mice were given an intradermal injection of cathepsin E (1 ng/site) and hind-paw scratching was counted for 60 min. Naltrexone hydrochloride (NTL, 1 mg/kg), naloxone hydrochloride (NLX, 1 mg/kg), and vehicle (VH) were injected subcutaneously 15 min before cathepsin E injection. A dotted line shows the number of scratching bouts after saline injection into the rostral back. Values represent the means ± S.E.M. for eight animals. *$P<0.05$ when compared with VH (Dunnett's test).

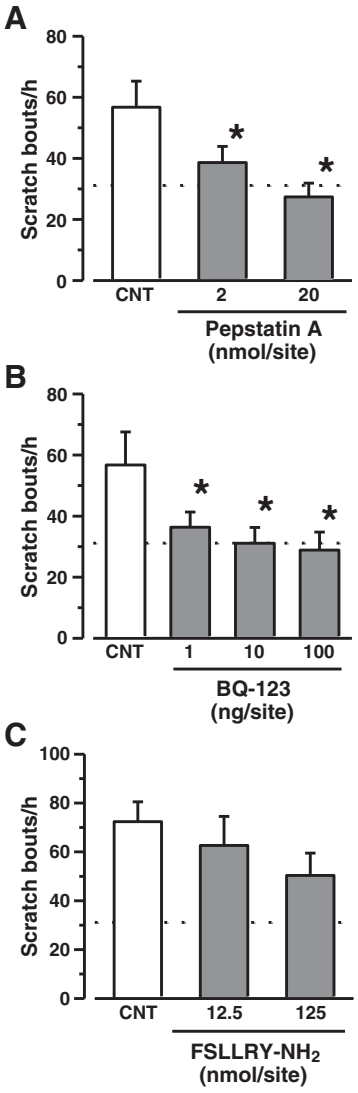

Fig. 4. Effects of pepstatin A, BQ-123, and FSLLRY-NH2 on scratching induced by cathepsin E. Mice were given an intradermal injection of cathepsin E (1 ng/site) and hind-paw scratching was counted for 60 min. Pepstatin A (A), BQ-123 (B), and FSLLRY-NH2 (C) were injected together with cathepsin E. Dotted lines show the number of scratching bouts after saline injection into the rostral back. CNT: control. Values represent the means ± S.E.M. for eight animals. *$P<0.05$ compared with the control (Dunnett's test).

NY, USA) and Alexa Flour 594-conjugated anti-mouse IgG antibody (Molecular Probes) for 2 h. Fluorescence signals were observed with a confocal microscope (Bio-Rad, Hercules, CA, USA).

2.6. Reverse transcription and polymerase chain reaction

Primary cultures of mouse keratinocytes were prepared as described previously (Andoh et al., 2009). Total RNA was extracted from keratinocytes using TRIzol® reagent (Invitrogen Co., Carlsbad, CA, USA), and treated with DNase I (Takara Bio Inc., Otsu, Japan). Total RNA (1 μg) was then reverse transcribed into cDNA using oligo$(dT)_{16}$ primer. PCR was performed as described previously (Andoh et al., 2004). The sequences of the primers used were as follows: preproendothelin-1 (sense), 5′-AACCTGGTCTGTGCTTTTTAGC-3′; preproendothelin-1 (antisense), 5′-TGGCCTGCCCAGACATATAT-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (sense), 5′-TTACTCCTTGGAGGCCACGT-3′; GAPDH (antisense), 5′-CCAAGGTCATCCATGACAAC-3′. Reaction products were separated on a 2% agarose gel and stained with ethidium bromide.

2.7. Data processing

Data are presented as means ± standard error of the mean (S.E.M.). Statistical significance was analyzed with Bonferroni's, Dunnett's, or Student's *t*-tests; $P<0.05$ was considered significant.

3. Results

3.1. Scratching behavior induced by cathepsin E

An intradermal injection of cathepsin E (1 ng/site) into the rostral back elicited hind-paw scratching at the injection site; the effect peaked in the first 10-min post-injection and almost subsided after 20 min (Fig. 1A). Cathepsin E at intradermal doses of 1–100 ng/site significantly increased scratching and a dose of 0.1 ng/site had no effect; the dose–response curve was bell-shaped with a peak effect at 1 ng/site (Fig. 1B). The scratch-inducing action of cathepsin E (1 ng/site) was almost abolished by its heat treatment (Fig. 1C).

To determine whether cathepsin E induces itching alone or itching together with pain, cathepsin E was injected intradermally into the cheek. Cathepsin E (0.2 and 1 ng/site) produced a dose-dependent increase in hind-paw scratching (itch-related response), but not forelimb wiping (pain-related response) (Fig. 2).

3.2. Effects of opioid antagonists on cathepsin E-induced scratching

Pretreatment with the opioid receptor antagonists naloxone hydrochloride (1 mg/kg) and naltrexone hydrochloride (1 mg/kg) significantly inhibited hind-paw scratching induced by cathepsin E (1 ng/site) (Fig. 3).

3.3. Effects of various agents on cathepsin E-induced scratching

When injected together with cathepsin E (1 ng/site), the aspartic protease inhibitor pepstatin A (2 and 20 nmol/site) significantly inhibited cathepsin E-induced scratching ($P<0.05$ each dose, Fig. 4A). Simultaneous administration of the ET_A endothelin receptor antagonist BQ-123 (1–100 ng/site) significantly inhibited cathepsin E (1 ng/site)-induced scratching ($P<0.05$ each dose, Fig. 4B). BQ-123 (100 ng/site) also significantly (Bonferroni's test) inhibited scratching induced by an intradermal injection of endothelin-1; the number of scratching bouts (per hour) was 26 ± 3, 142 ± 20, and 30 ± 4 ($n=8$ each) after injection of the vehicle, endothelin-1 (30 pmol), and endothelin-1 (30 pmol) + BQ-123, respectively. The proteinase-activated receptor-2 (PAR_2) antagonist FSLLRY-NH_2 (12.5 and 125 nmol/site) did not

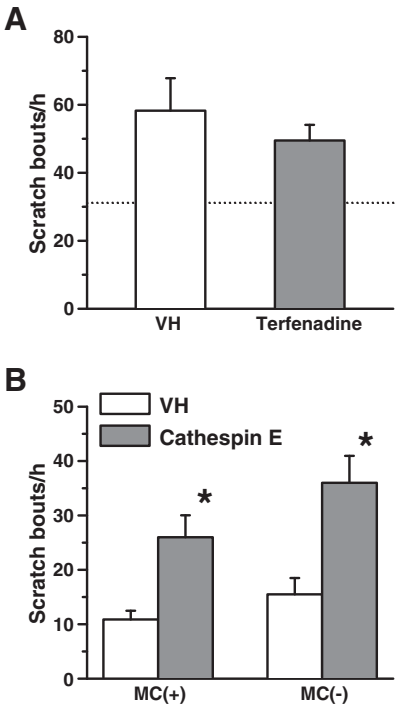

Fig. 5. Effects of terfenadine and mast cell deficiency on scratching induced by cathepsin E. Mice were given an intradermal injection of cathepsin E (1 ng/site) and hind-paw scratching was counted for 60 min. (A) Terfenadine and vehicle (VH) were administered orally to ICR mice 30 min before the injection. A dotted line shows the number of scratching bouts after saline injection into the rostral back. (B) MC(−) and MC(+) show the data obtained from mast cell-deficient and wild-type mice, respectively. Values represent the means ± S.E.M. for eight animals. *$P<0.05$ compared with VH (Bonferroni's test).

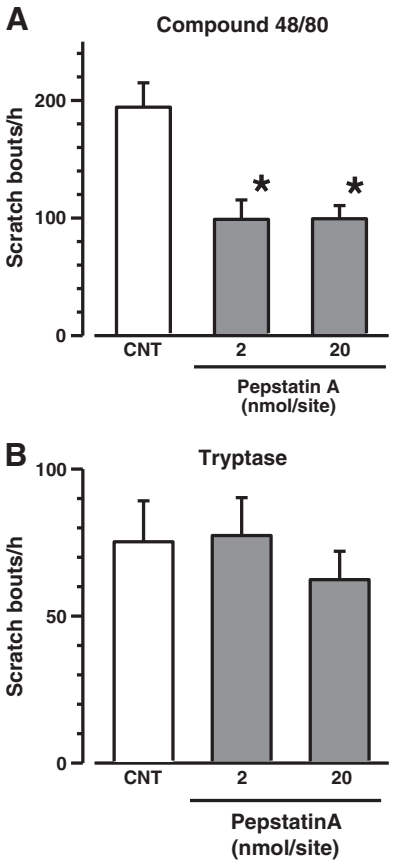

Fig. 6. Effects of pepstatin A on scratching induced by compound 48/80 and tryptase. Mice were given an intradermal injection of compound 48/80 (A, 10 μg/site) or tryptase (B, 0.1 ng/site) and hind-paw scratching was counted for 60 min. Pepstatin A was injected together with pruritogens. Values represent the means ± S.E.M. for eight animals. *$P<0.05$ compared with the control (Dunnett's test).

significantly inhibit cathepsin E-induced scratching, although there was a tendency toward a decrease (Fig. 4C).

3.4. Effect of H_1 histamine receptor antagonist and mast cell deficiency on scratching induced by cathepsin E and endothelin-1

The H_1 histamine receptor antagonist terfenadine (30 mg/kg) did not suppress scratching induced by an intradermal injection of cathepsin E (1 ng/site) (Fig. 5A). In both mast cell-deficient WBB6F1 W/W^v mice and their normal WBB6F1+/+ littermates, an intradermal injection of cathepsin E (1 ng/site) significantly increased scratching compared with the vehicle; mast-cell deficiency did not inhibit cathepsin E-induced scratching (Fig. 5B). Scratching induced by endothelin-1 (30 pmol/site) was not inhibited by pretreatment with terfenadine (30 mg/kg); the number of scratching bouts (per hour) was 150 ± 21 and 160 ± 29 ($n=8$ each) in the groups administered vehicle and terfenadine, respectively.

3.5. Effects of pepstatin A on compound 48/80- and tryptase-induced scratching

Simultaneous injections of pepstatin A (2 and 20 nmol/site) partially but significantly inhibited scratching induced by an intradermal injection of compound 48/80 (10 μg/site) (Fig. 6A). Pepstatin A at the same doses did not inhibit scratching induced by an intradermal injection of tryptase (0.1 ng/site) (Fig. 6B).

3.6. Effect of cathepsin E on the level of endothelin-1 in the skin

An intradermal injection of cathepsin E (1 ng/site) significantly increased the level of endothelin-1 in the skin at the injection site compared with saline injection (Fig. 7).

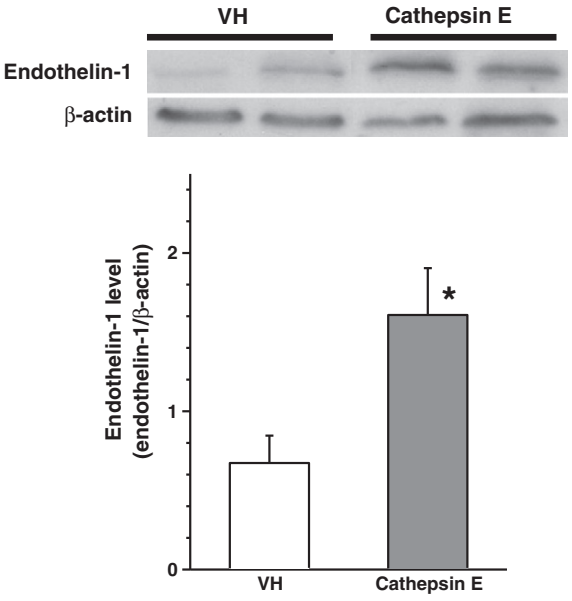

Fig. 7. Cathepsin E increased the intracutaneous level of endothelin-1. Cathepsin E (1 ng/site) or vehicle (VH) was injected intradermally into the rostral back, the skin of which was isolated 10 min later for western blot determination of endothelin-1; representative western blot data (two samples each group) are shown in the upper panel. The level of endothelin-1 was normalized to that of β-actin. Values represent the means ± S.E.M. for eight animals. *$P<0.05$ (Student's t-test).

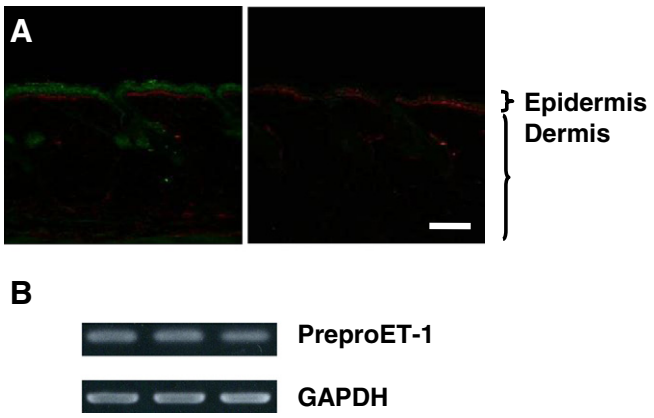

Fig. 8. Expression of endothelin-1 in the skin and preproendothelin-1 mRNA in primary cultures of keratinocytes. (A) Double staining for endothelin-1 and keratin 14 in the skin. Left, anti-endothelin-1 antibody; right, pre-absorbed anti-endothelin-1 antibody; green, endothelin-1-like immunoreactivity; red, keratin 14-like immunoreactivity. Scale bar = 100 μm. (B) Expression of preproendothelin-1 mRNA in primary cultures of keratinocytes. Samples from three different culture dishes were assayed.

3.7. Distribution of endothelin-1 in the skin and expression of preproendothelin-1 mRNA in primary cultures of mouse keratinocytes

Endothelin-1-like immunoreactivity was present in the epidermis; staining for keratin 14, a marker of epidermal basal cells (Aden et al., 2008), made clear the localization of endothelin-1 in the prickle and granular cell layers (Fig. 8A, B). Reverse transcriptase and polymerase chain reaction showed the expression of preproendothelin-1 mRNA in primary cultures of mouse keratinocytes (Fig. 8B).

4. Discussion

Cathepsin E elicited hind-paw scratching after intradermal injection into the rostral back and cheek of mice, suggesting that cathepsin E is pruritogenic. Cathepsin E-induced scratching was inhibited by the opioid receptor antagonists naltrexone and naloxone. In view of the findings that opioid antagonists inhibit itching and/or scratching in patients with pruritic diseases, such as cholestasis, chronic urticaria, and atopic dermatitis in humans (Bergasa et al., 1995; Monroe, 1989) as well as in many mouse itch models (Andoh et al., 1998, 2004, 2009; Miyamoto et al., 2002; Ohtsuka et al., 2001; Yamaguchi et al., 1999, 2001), these results support the idea that cathepsin E-induced scratching is an itch-related response. An intradermal injection of cathepsin E into the cheek elicited hind-paw scratching but not forelimb wiping, suggesting that intradermal cathepsin E selectively induces itch and does not cause pain. Itching and scratching have been shown to be induced by serine proteases (e.g., tryptase, trypsin, chymase, and kallikrein) and cysteine proteases (e.g., muconain and cathepsin S) in humans and/or rodents (Costa et al., 2008; Hägermark, 1974; Hägermark et al., 1972; Reddy et al., 2008, 2010; Ui et al., 2006). This is the first report of the pruritogenic action of an aspartic protease.

Itching/scratching induced by chymase and trypsin may be mediated at least in part by histamine and/or other mediators released from mast cells in humans and mice (Costa et al., 2008; Hägermark et al., 1972). In contrast, cathepsin E-induced scratching was not inhibited by the H_1 histamine receptor antagonist terfenadine at an oral dose of 30 mg/kg, which significantly suppresses scratching induced by histamine and passive cutaneous anaphylaxis in mice (Inagaki et al., 1999; Ohtsuka et al., 2001). In addition, cathepsin E-elicited scratching was similar between WBB6F1 W/W^v (mast cell-deficient) and WBB6F1+/+ (normal control) mice. These results suggest that mast cell mediators, including histamine, do not play an important role in cathepsin E-induced scratching.

Heat treatment of cathepsin E and simultaneous administration of the aspartic protease inhibitor pepstatin A (Chain et al., 2005) almost abolished cathepsin E-induced scratching, suggesting that enzyme activity is responsible for its pruritogenic activity. The enzyme activity of cathepsin E is invariable over a low pH range (3.0–4.5) and diminishes above pH 5, but a significant level of cathepsin E activity is observed even at pH 7.0 in the presence of adenosine 5′-triphosphate (Thomas et al., 1989). Since the pH values of the cathepsin E solution administered and the murine dermis were 6.25 and 6.90 ± 0.02 ($n = 6$), respectively (our preliminary experiments), cathepsin E might function as aspartic protease in the presence of intra- and extracellular adenosine 5′-triphosphate.

The PAR_2 receptors are responsible for itching/scratching induced by trypsin-type serine proteases such as trypsin and tryptase (Costa et al., 2008; Ui et al., 2006). The PAR_2 and PAR_4 receptors may be involved in itching induced by cysteine proteases such as mucunain and cathepsin S (Reddy et al., 2008, 2010). In contrast, the PAR_2 antagonist FSLLRY did not attenuate scratching induced by cathepsin E. Thus, the PAR_2 receptors may not play a key role in cathepsin E-induced scratching.

Pepstatin A significantly but partly inhibited scratching induced by compound 48/80, which degranulates mast cells with them intact (Ellis et al., 1970), at doses that inhibited cathepsin E-induced, but not tryptase-induced, scratching. These results suggest that aspartic protease, probably cathepsin E, is involved at least in part in mast cell-dependent itching. Thus, mast cells release multiple itch mediators, such as histamine, tryptase, and cathepsin E.

Scratching induced by intradermal cathepsin E was found to be inhibited by the endothelin ET_A receptor antagonist BQ-123. An intradermal injection of endothelin-1 alone in mouse elicits scratching, which is suppressed by BQ-123 (McQueen et al., 2007; Trentin et al., 2006, present experiments). Thus, it is suggested that endothelin ET_A receptor, and probably endothelin-1 generated from big-endothelin-1 by cathepsin E, are involved in cathepsin E-induced scratching. The result that terfenadine (30 mg/kg) did not affect scratching induced cathepsin E and endothelin-1 is consistent with the above idea. Cathepsin E processes big-endothelin-1 to generate endothelin-1 (Lees et al., 1990) and an intradermal injection of cathepsin E increased the intracutaneous level of endothelin-1. Endothelin-1 was present in the prickle and granular cell layers of the epidermis and preproendothelin-1 mRNA in primary cultures of epidermal keratinocytes. Therefore, a possible origin of big-endothelin-1 is epidermal keratinocytes. Therefore, it is possible that cathepsin E in the epidermis is involved in itching. Since cathepsin E is present in the prickle and granular cell layers of epidermis (Hara et al., 1993), in addition to mast-cell cathepsin E, keratinocyte cathepsin E may also be involved in itching. An intradermal injection of the content of edema after immediate allergy causes scratching in naïve mice (Nakano et al., 2008), another possible source of big-endothelin-1 is blood plasma. However, plasma concentrations of big-endothelin-1 and endothelin-1 are less than 1 fmol/ml in rodents (Lovric-Bencic et al., 2004), which are far less than pruritogenic doses of endothelin-1 (0.01–10 nmol/ml) in mice (McQueen et al., 2007; Trentin et al., 2006; present experiments). Therefore, blood plasma may not be an important source of pruritogenic endothelin-1 and big-endothelin-1.

Endothelin-1 has high binding affinity for the endothelin ET_A and ET_B receptor subtypes (Masaki, 2004), and the ET_A receptor subtype may play a key role in endothelin-1-induced scratching in mice (McQueen et al., 2007; Trentin et al., 2006; present experiment). The adenylyl cyclase-protein kinase C pathway has been shown to be involved in ET_A receptor-mediated endothelin-1-induced scratching (Liang et al., 2010). ET_A, but not ET_B, receptors are expressed in primary sensory neurons (Pomonis et al., 2001), and endothelin-1 activity may be mediated by the potentiation of co-expressed transient receptor potential vanilloid 1 (Plant et al., 2007). The role of the ET_B receptor in endothelin-1-associated scratching is controversial (McQueen et al., 2007; Trentin et al., 2006).

In summary, intradermal cathepsin E has pruritogenic activity, which is at least partly mediated by the production of endothelin-1. This is the first report of a pruritogenic effect of an aspartic protease with the involvement of generation of a pruritic peptide as part of its effect.

Acknowledgment

This study was supported in part by a Grant-in-Aid for Young Scientists (B) #22790063 and for Scientific Research (B) #23390153 from the Ministry of Education, Culture, Sports, Science and Technology, Japan and by Grants for Health Science from the Health, Labor and Welfare Ministry, Japan.

References

Aden, N., Shiwen, X., Aden, D., Black, C., Nuttall, A., Denton, C.P., Leask, A., Abraham, D., Stratton, R., 2008. Proteomic analysis of scleroderma lesional skin reveals activated wound healing phenotype of epidermal cell layer. Rheumatology (Oxford) 47, 1754–1760.

Andoh, T., Nagasawa, T., Satoh, M., Kuraishi, Y., 1998. Substance P induction of itch-associated response mediated by cutaneous NK_1 tachykinin receptors in mice. J. Pharmacol. Exp. Ther. 286, 1140–1145.

Andoh, T., Yageta, Y., Takeshima, H., Kuraishi, Y., 2004. Intradermal nociceptin elicits itch-associated responses through leukotriene B_4 in mice. J. Invest. Dermatol. 123, 196–201.

Andoh, T., Saito, A., Kuraishi, Y., 2009. Leukotriene B_4 mediates sphingosylphosphorylcholine-induced itch-associated responses in mouse skin. J. Invest. Dermatol. 129, 2854–2860.

Arthur, R.P., Shelley, W.B., 1955. The role of proteolytic enzymes in the production of pruritus in man. J. Invest. Dermatol. 25, 341–346.

Bergasa, N.V., Alling, D.W., Talbot, T.L., Swain, M.G., Yurdaydin, C., Turner, M.L., Schmitt, J.M., Walker, E.C., Jones, E.A., 1995. Effects of naloxone infusions in patients with the pruritus of cholestasis. A double-blind, randomized, controlled trial. Ann. Intern. Med. 123, 161–167.

Chain, B.M., Free, P., Medd, P., Swetman, C., Tabor, A.B., Terrazzini, N., 2005. The expression and function of cathepsin E in dendritic cells. J. Immunol. 174, 1791–1800.

Costa, R., Marotta, D.M., Manjavachi, M.N., Fernandes, E.S., Lima-Garcia, J.F., Paszcuk, A.F., Quintão, N.L., Juliano, L., Brain, S.D., Calixto, J.B., 2008. Evidence for the role of neurogenic inflammation components in trypsin-elicited scratching behaviour in mice. Br. J. Pharmacol. 154, 1094–1103.

Davies, M.G., Marks, R., Horton, R.J., Storari, F.E., 1979. The efficacy of histamine antagonists as antipruritics in experimentally induced pruritus. Arch. Dermatol. Res. 266, 117–120.

Ellis, H.V., Johnson, A.R., Moran, N.C., 1970. Selective release of histamine from rat mast cells by several drugs. J. Pharmacol. Exp. Ther. 175, 627–631.

Hägermark, Ö., 1973. Influence of antihistamines, sedatives, and aspirin on experimental itch. Acta Derm. Venereol. 53, 363–368.

Hägermark, Ö., 1974. Studies on experimental itch induced by kallikrein and bradykinin. Acta Derm. Venereol. 54, 397–400.

Hägermark, Ö., Rajka, G., Bergvist, U., 1972. Experimental itch in human skin elicited by rat mast cell chymase. Acta Derm. Venereol. 52, 125–128.

Hara, K., Fukuyama, K., Saka, H., Yamamoto, K., Epstein, W.L., 1993. Purification and immunohistochemical localization of aspartic proteinases in rat epidermis. J. Invest. Dermatol. 100, 394–399.

Henningsson, F., Yamamoto, K., Saftig, P., Reinheckel, T., Peters, C., Knight, S.D., Pejler, G., 2005. A role for cathepsin E in the processing of mast-cell carboxypeptidase A. J. Cell Sci. 118, 2035–2042.

Inagaki, N., Nakamura, N., Nagao, M., Musoh, K., Kawasaki, H., Nagai, H., 1999. Participation of histamine H_1 and H_2 receptors in passive cutaneous anaphylaxis-induced scratching behavior in ICR mice. Eur. J. Pharmacol. 367, 361–371.

Kageyama, T., Ichinose, M., Yonezawa, S., 1995. Processing of the precursors to neurotensin and other bioactive peptides by cathepsin E. J. Biol. Chem. 270, 19135–19140.

Kuraishi, Y., Nagasawa, T., Hayashi, K., Satoh, M., 1995. Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. Eur. J. Pharmacol. 275, 229–233.

Lees, W.E., Kalinka, S., Meech, J., Capper, S.J., Cook, N.D., Kay, J., 1990. Generation of human endothelin by cathepsin E. FEBS Lett. 273, 99–102.

Liang, J., Kawamata, T., Ji, W., 2010. Molecular signaling of pruritus induced by endothelin-1 in mice. Exp. Biol. Med. (Maywood) 235, 1300–1305.

Lovric-Bencic, M., Sikiric, P., Hanzevacki, J.S., Seiwerth, S., Rogic, D., Kusec, V., Aralica, G., Konjevoda, P., Batelja, L., Blagaic, A.B., 2004. Doxorubicine-congestive heart failure-increased big endothelin-1 plasma concentration: reversal by amlodipine, losartan, and gastric pentadecapeptide BPC157 in rat and mouse. J. Pharmacol. Sci. 95, 19–26.

Macfarlane, S.R., Seatter, M.J., Kanke, T., Hunter, G.D., Plevin, R., 2001. Proteinase-activated receptor. Pharmacol. Rev. 53, 254–282.

Maekawa, T., Nojima, H., Kuraishi, Y., 2000. Itch-associated responses of afferent nerve innervating the murine skin: different effects of histamine and serotonin in ICR and ddY mice. Jpn. J. Pharmacol. 84, 462–466.

Masaki, T., 2004. Historical review: endothelin. Trends Pharmacol. Sci. 25, 219–224.

McQueen, D.S., Noble, M.A., Bond, S.M., 2007. Endothelin-1 activates ET_A receptors to cause reflex scratching in BALB/c mice. Br. J. Pharmacol. 151, 278–284.

Metcalfe, D.D., Baram, D., Mekori, Y.A., 1997. Mast cells. Physiol. Rev. 77, 1033–1079.

Miyamoto, T., Nojima, H., Shinkado, T., Nakahashi, T., Kuraishi, Y., 2002. Itch-associated response induced by experimental dry skin in mice. Jpn. J. Pharmacol. 88, 285–292.

Monroe, E.W., 1989. Efficacy and safety of nalmefene in patients with severe pruritis caused by chronic urticaria and atopic dermatitis. J. Am. Acad. Dermatol. 21, 135–136.

Nakano, T., Andoh, T., Sasaki, A., Nojima, H., Kuraishi, Y., 2008. Different roles of capsaicin-sensitive and H_1 histamine receptor-expressing sensory neurons in itch of mosquito allergy in mice. Acta Derm. Venereol. 88, 449–454.

Ohtsuka, E., Kawai, S., Ichikawa, T., Nojima, H., Kitagawa, K., Shirai, Y., Kamimura, K., Kuraishi, Y., 2001. Roles of mast cells and histamine in mosquito bite-induced allergic itch-associated responses in mice. Jpn. J. Pharmacol. 86, 97–105.

Plant, T.D., Zöllner, C., Kepura, F., Mousa, S.S., Eichhorst, J., Schaefer, M., Furkert, J., Stein, C., Oksche, A., 2007. Endothelin potentiates TRPV1 via ET_A receptor-mediated activation of protein kinase C. Mol. Pain 3, 35.

Pomonis, J.D., Rogers, S.D., Peters, C.M., Ghilardi, J.R., Mantyh, P.W., 2001. Expression and localization of endothelin receptors: implications for the involvement of peripheral glia in nociception. J. Neurosci. 21, 999–1006.

Reddy, V.B., Iuga, A.O., Shimada, S.G., LaMotte, R.H., Lerner, E.A., 2008. Cowhage-evoked itch is mediated by a novel cysteine protease: a ligand of protease-activated receptors. J. Neurosci. 28, 4331–4335.

Reddy, V.B., Shimada, S.G., Sikand, P., Lamotte, R.H., Lerner, E.A., 2010. Cathepsin S elicits itch and signals via protease-activated receptors. J. Invest. Dermatol. 130, 1468–1470.

Shimada, S.G., LaMotte, R.H., 2008. Behavioral differentiation between itch and pain in mouse. Pain 139, 681–687.

Steinhoff, M., Neisius, U., Ikoma, A., Fartasch, M., Heyer, G., Skov, P.S., Luger, T.A., Schmelz, M., 2003. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. J. Neurosci. 23, 6176–6180.

Thomas, D.J., Richards, A.D., Jupp, R.A., Ueno, E., Yamamoto, K., Samloff, I.M., Dunn, B.M., Kay, J., 1989. Stabilisation of cathepsin E by ATP. FEBS Lett. 243, 145–148.

Trentin, P.G., Fernandes, M.B., D'Orléans-Juste, P., Rae, G.A., 2006. Endothelin-1 causes pruritus in mice. Exp. Biol. Med. (Maywood) 231, 1146–1151.

Tsujii, K., Andoh, T., Lee, J.B., Kuraishi, Y., 2008. Activation of proteinase-activated receptors induces itch-associated response through histamine-dependent and -independent pathways in mice. J. Pharmacol. Sci. 108, 385–388.

Tsujii, K., Andoh, T., Ui, H., Lee, J.B., Kuraishi, Y., 2009. Involvement of tryptase and proteinase-activated receptor-2 in spontaneous itch-associated response in mice with atopy-like dermatitis. J. Pharmacol. Sci. 109, 388–395.

Ui, H., Andoh, T., Lee, J.B., Nojima, H., Kuraishi, Y., 2006. Potent pruritogenic action of tryptase mediated by PAR-2 receptor and its involvement in anti-pruritic effect of nafamostat mesilate in mice. Eur. J. Pharmacol. 530, 172–178.

Watanabe, N., Tomimori, Y., Saito, K., Miura, K., Wada, A., Tsudzuki, M., Fukuda, Y., 2002. Chymase inhibitor improves dermatitis in NC/Nga mice. Int. Arch. Allergy Immunol. 128, 229–234.

Yamaguchi, T., Nagasawa, T., Satoh, M., Kuraishi, Y., 1999. Itch-associated response induced by intradermal serotonin through 5-HT_2 receptors in mice. Neurosci. Res. 35, 77–83.

Yamaguchi, T., Maekawa, T., Nishikawa, Y., Nojima, H., Kaneko, M., Kawakita, T., Miyamoto, T., Kuraishi, Y., 2001. Characterization of itch-associated responses of NC mice with mite-induced chronic dermatitis. J. Dermatol. Sci. 25, 20–28.

Zaidi, N., Kalbacher, H., 2008. Cathepsin E: a mini review. Biochem. Biophys. Res. Commun. 367, 517–522.