See related commentary on pg 1857

Thromboxane A_2 Induces Itch-Associated Responses through TP Receptors in the Skin in Mice

Tsugunobu Andoh[1,5], Yumi Nishikawa[1,5], Tomomi Yamaguchi-Miyamoto[1], Hiroshi Nojima[2], Shu Narumiya[3] and Yasushi Kuraishi[1,4]

Thromboxane A_2 (TXA_2), a metabolite of arachidonic acid produced by cyclooxygenase and thromboxane synthase, is thought to participate in chronic dermatitis. This study investigated the involvement of TXA_2 in cutaneous itch. An intradermal injection of U-46619, a stable analogue of TXA_2, elicited scratching, an itch-associated response, in mice. Dose–response curve was bell shaped with a maximum effect at 10 nmol per site. The action of U-46619 was inhibited by a coinjection of the TP antagonist ONO-3708 and was abolished by TP receptor deficiency. TP receptor was mainly expressed in nerve fiber in the skin and keratinocytes. Thromboxane synthase was also expressed in keratinocytes. U-46619 increased intracellular Ca^{2+} ion concentration in primary cultures of dorsal root ganglion neurons and keratinocytes. The results suggest that TXA_2 synthesized by keratinocytes acts as an itch mediator. It may elicit itch through the activation of TP receptors on primary afferents and keratinocytes; keratinocytes may produce itch mediators including TXA_2. Thus, thromboxane synthase inhibitor and TP receptor antagonists will be candidates for antipruritic medicines.

Journal of Investigative Dermatology (2007) **127,** 2042–2047; doi:10.1038/sj.jid.5700810; published online 12 April 2007

INTRODUCTION

Itch is a distressing symptom associated with a variety of peripheral and systemic disorders. This sensation provokes a desire to scratch and makes cutaneous symptom worse. H_1 histamine receptor antagonists are the drugs of first choice for the treatment of itch, but many pruritic diseases (e.g., atopic dermatitis) except acute urticaria respond poorly to the H_1 receptor antagonists (Wahlgren, 1991). Thus, the precise mechanisms and mediators of itch in most pruritic diseases are unclear.

Glucocorticoids suppress itch of severe chronic skin diseases (Felix and Shuster, 1975). Glucocorticoids inhibit the phospholipase A_2 (Piltch *et al.*, 1989) and regulate the expression of cyclooxygenase-2, but not cyclooxygenase-1 (Zhang *et al.*, 1999). Therefore, arachidonic acid metabolites may be involved in the induction and/or enhancement of itch. In human subjects, prostaglandin E_2 is a weak pruritogen (Hägermark and Strandberg (1977)) and prolongs experimentally induced itch (Fjellner and Hägermark 1979; Hägermark and Strandberg (1977)). In addition, itching of patients with polycythemia vera is suppressed by the cyclooxygenase inhibitor aspirin (Fjellner and Hägermark, 1979). In animal experiments, an intradermal injection of prostaglandin E_2 alone does not elicit itch-associated response (Andoh and Kuraishi, 1998).

Thromboxane A_2 (TXA_2) is produced by catalysis with cyclooxygenase and thromboxane synthase, and is altered spontaneously to inactive TXB_2. TXA_2 exerts its actions through a G-protein-coupled receptor, termed prostanoid receptor (TP) (Narumiya *et al.*, 1999). TXA_2 is produced by the platelets and affects the cardiovascular system (FitzGerald *et al.*, 1987), including platelet aggregation (Svensson *et al.*, 1976). The serum concentration of TXB_2 is increased in patients with pruritic diseases (Marks *et al.*, 1984; Mysliwiec *et al.*, 1985; Grekas *et al.*, 1989; Veale *et al.*, 1994). It was also increased in the skin of NC mice, which had chronic dermatitis and showed chronic itch-associated behavior (Andoh and Kuraishi, unpublished observation). However, it is unclear whether TXA_2 is involved in cutaneous itch. In this study, we investigated whether TXA_2 and TP receptor would be involved in cutaneous itch.

RESULTS

U-46619 elicits scratching through TP receptor

As TXA_2 is very unstable, we used U-46619, a stable analoge of TXA_2. An intradermal injection of U-46619 (10 nmol per site), but not vehicle (0.2% ethanol in saline), markedly elicited scratching of the injected site by the hind paw in Institute for Cancer Research (ICR) mice (Figure 1). The effect

[1]*Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan;* [2]*Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ohu University, Koriyama, Japan;* [3]*Department of Pharmacology, Graduate School of Medicine, Kyoto University, Kyoto, Japan and* [4]*21st Century COE Program, University of Toyama, Toyama, Japan*

[5]*These authors contributed equally to this work*

Correspondence: Dr Yasushi Kuraishi, Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-1094, Japan.
E-mail: kuraisiy@pha.u-toyama.ac.jp

Abbreviations: mKGM, mouse keratinocyte growth medium; TP, TP prostanoid receptor, TXA_2, thromboxane A_2; TXB_2, thromboxane B_2

Received 27 November 2006; revised 10 January 2007; accepted 30 January 2007; published online 12 April 2007

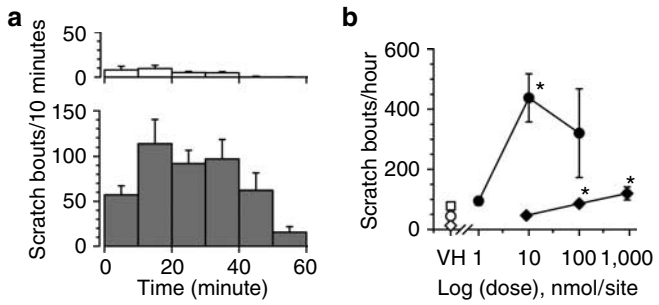

Figure 1. Scratching after intradermal injection of the TP receptor agonist U-44619 in ICR mice. **(a)** Time course of scratching after vehicle (upper panel, 0.2% ethanol in saline) and U-46619 (lower panel, 10 nmol per site) injection. **(b)** Dose–response curves for the scratch-inducing effects of U-46619 and histamine. ICR mice were given an intradermal injection of U-46619, histamine, or vehicle (VH; ○, 0.2% ethanol in saline; □, 2% ethanol in saline; △, saline). Values represent the means ± SEM for eight animals. *$P<0.05$ when compared with VH for each agent (Dunnett's multiple comparisons).

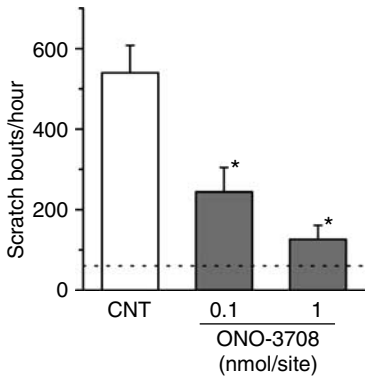

Figure 2. Suppressive effects of the TP receptor antagonist ONO-3708 on U-46619-induced scratching in ICR mice. U-46619 (10 nmol per site) was injected intradermally alone (CNT) or together with ONO-3708 into ICR mice. Dashed line shows data obtained from mice injected intradermally with vehicle (0.2% ethanol in saline). Values represent the means ± SEM for eight animals. *$P<0.05$ when compared with CNT (Dunnett's multiple comparisons).

peaked during the second 10-minutes period and almost completely subsided by 60 minute (Figure 1a). U-46619 at intradermal doses of 1–100 nmol per site elicited scratching; the dose–response curve was bell-shaped with a peak effect at 10 nmol per site (Figure 1b). U-46619 at a dose of 100 nmol/site elicited abnormal behaviors such as writhing and crouching in three out of eight mice tested. Intradermal injections of histamine at doses of 9–900 nmol per site elicited scratching in a dose-dependent manner (Figure 1b). The number of scratching induced by U-46619 (10 nmol per site) was 3.6 times more than that by histamine (900 nmol per site). Intraplantar injections of U-46619 (4 and 10 nmol per site) did not produce licking behavior, a pain-related response (data not shown).

Scratching elicited by U-46619 at a dose of 10 nmol per site was dose dependently suppressed by coinjection of the TP receptor antagonist ONO-3708 at doses of 0.1–1 nmol per site (Figure 2). An intradermal injection of U-46619 (10 nmol per site) did not elicit scratching in $TP^{-/-}$ mice, although it elicited substantial scratching in wild-type (C57BL/6) mice (Figure 3).

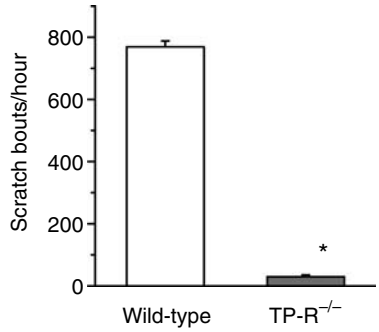

Figure 3. Deficiency in TP thromboxane A_2 receptor abolishes scratching induced by intradermal injection of U-46619 in mice. U-46619 (10 nmol per site) was injected intradermally into wild-type (C57BL/6 strain) and TP receptor-deficient mice ($TP\text{-}R^{-/-}$). Values represent the means ± SEM for eight animals. *$P<0.05$ when compared with wild type (Student's *t*-test).

Distribution of thromboxane synthase and TP receptor in the skin

Immunohistochemical staining showed that thromboxane synthase was expressed only in the epidermal keratinocytes; the distinct immunoreactivity was not observed in the dermis. (Figure 4a). On the other hand, TP receptors were localized mainly in the keratinocytes, and also in the nerve fibers positive for protein gene product 9.5, a pan-neuronal marker, especially in the epidermis (Figure 4b). Figure 4c shows frequency distribution of TP receptor-immunoreactive neurons in the dorsal root ganglia. When 1,133 cultured dorsal root ganglion neurons were examined, 597 neurons (53%) were to be found immunoreactive for TP receptors. Four hundred and twenty-four (78%) out of 544 small-size neurons ($\leqslant 15\,\mu$m in diameter), 160 (48%) out of 331 medium-sized neurons (>15 and $\leqslant 25\,\mu$m), and 13 (5%) out of 258 large-sized neurons ($\geqslant 25\,\mu$m) were immunoreactive for TP receptor. Seventy-one percent of 597 TP-immunoreactive neurons were small in size.

U-46619 activates dorsal root ganglion neurons and keratinocytes through TP receptor

In the above behavioral experiments, an intradermal injection of U-46619 (10 nmol/50 μl = 200 μM) elicited scratching, which was inhibited by ONO-3708 (1 nmol/50 μl = 20 μM). Therefore, in this series of *in vitro* experiments, U-46619 and ONO-3708 were administered at the final concentrations of 200 and 20 μM, respectively. Figure 5a shows a typical example of the response of primary culture of mouse dorsal root ganglion neuron to U-46619. It was markedly increased by the concentration of intracellular Ca^{2+} ions, which was significantly suppressed in the presence of ONO-3708 (Figure 5a and b). The effect peaked around 1 minute after administration and almost subsided by 8 minutes (Figure 5a). Similar results were also obtained from primary culture of mouse epidermal keratinocytes. U-46619 markedly increased

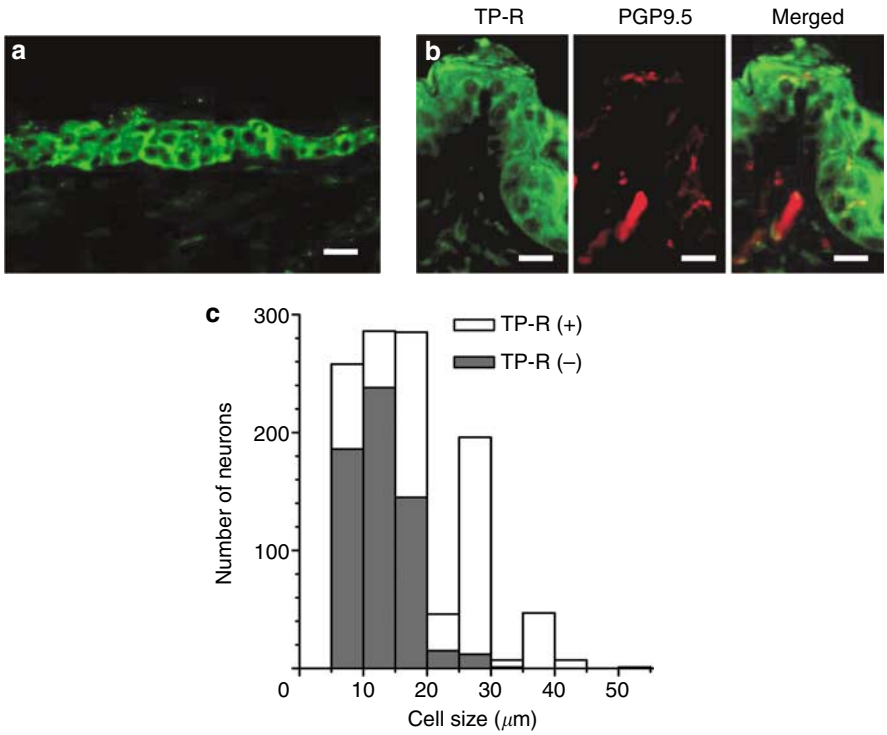

Figure 4. Expression of thromboxane synthase and TP receptor in the mouse skin and dorsal root ganglion neurons. (**a**) Thromboxane synthase immunoreactivity in the skin. It was observed in the epidermal keratinocytes. Bar = 20 μm. **(b)** TP receptor (TP-R) and PGP9.5 immunoreactivities in the skin. TP-R (green) was observed in the epidermal keratinocytes. Scale bar = 20 μm. **(c)** Frequency distribution of TP-R-positive neurons in the dorsal root ganglion. Closed and open columns indicate TP-R-positive ($n = 597$) and negative neurons ($n = 536$), respectively.

the concentration of intracellular Ca^{2+} ions, but not in the presence of ONO-3708, in keratinocytes (Figure 5c and d). The effect peaked around 1 minute after administration and almost subsided by 8 minutes (Figure 5c).

Administration of vehicles for ONO-3708 and U-46619 did not affect the concentration of intracellular Ca^{2+} ions in the neurons and keratinocytes (data not shown).

DISCUSSION

An intradermal injection of the TP receptor agonist U-46619 elicited scratching of the injection site in mice. U-46619-elicited scratching was abolished by deficiency in TP receptor and was inhibited by the TP receptor antagonist ONO-3708. ONO-3708 inhibits the receptor binding of U-46619 but not of leukotriene D_4, TXB_2, and prostaglandins D_2 and E_1 (Narumiya *et al.* 1986; Nagai *et al.*, 1991). Therefore, it is suggested that the action of U-46619 on the skin elicits itch-related response through the stimulation of TP receptors. The effect of U-46619 peaked at a dose of 10 nmol per site, which was 10–100 times lower than those of substance P (Andoh *et al.*, 1998), histamine (Andoh and Kuraishi (2002)), serotonin (Yamaguchi *et al.*, 1999), and nociceptin (Andoh *et al.*, 2004), and was about three times more than that of leukotriene B_4 (Andoh and Kuraishi, 1998). The results, taken together, suggest that TXA_2 is an itch mediator in the skin. The number of scratching after a 100 nmol/site dose was less than that after a 10 nmol/site dose. Systemic effects of U-46619 at the highest dose tested might suppress itch-related behavior because it elicited abnormal behaviors such as writhing and crouching.

TXA_2 is synthesized from prostaglandin H_2, a metabolite of arachidonic acid, by thromboxane synthase (Needleman *et al.*, 1976). In the normal skin of the mice, thromboxane synthase was exclusively expressed in the keratinocytes in the epidermis, suggesting that the keratinocytes produce and release TXA_2 in the skin. In human subjects, oral aspirin was reported not to affect itch of some pruritic diseases such as chronic eczema, psoriasis, and biliary cirrhosis at an analgesic dose (Daly and Shuster, 1986). On the other hand, topical aspirin (twice daily at least for 2 weeks) was shown to reduce pruritus of lichen simplex chronicus (Yosipovitch *et al.* (2001)). There is no evidence that the inhibition of cyclooxygenase is responsible for the anti-pruritic action of aspirin, but it is interesting to investigate changes in the concentration of TXA_2 and the expression level of thromboxane synthase in the epidermis of the affected skin. We do not deny the possibility that TXA_2 released from the platelets and/or inflammatory cells including eosinophils (Kroegel *et al.*, 1994) is also involved in pruritus. For example, oral aspirin (1.5 g daily for 1 week) alleviates the pruritus of polycythemia vera (Fjellner and Hägermark, 1979). Proliferation of all hematopoietic bone marrow cells including platelets abnormally increased in this disorder, and the pruritus was suggested to be mediated by prostaglandin E_2 and serotonin

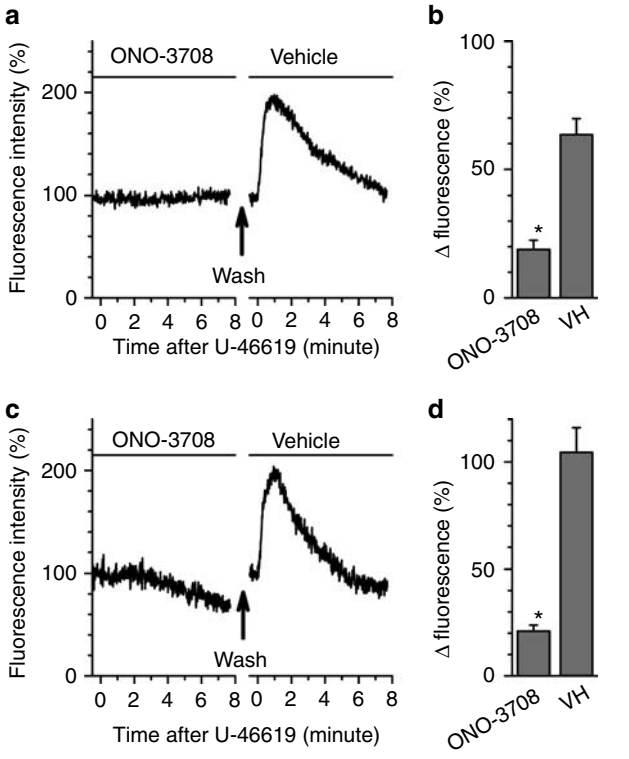

Figure 5. U-46619-induced increase in the concentration of intracellular Ca^{2+} ions in the cultured mouse dorsal root ganglion neurons and keratinocytes. Typical examples of time-course of U-46619-induced changes in the concentration of intracellular Ca^{2+} ions (fluorescence) in the dorsal root ganglion neuron (**a**) and keratinocytes **(c)** Maximal change in the U-46619-induced increase of intracellular Ca^{2+} ions in the dorsal root ganglion neurons (**b**, $n=20$) and keratinocytes (**d**, $n=33$). Primary cultures of mouse dorsal root ganglion neurons and keratinocytes were preloaded with fluo-3. U-46619 was administered to the culture medium at the final concentration of 200 μM. ONO-3708 (final concentration of 10 μM) and vehicle (VH) were administered 5 minutes before U-46619. Values represent the means $\pm$ SEM. *$P<0.05$ when compared with VH.

released from platelets. However, as platelets also synthesize and release TXA_2, it seems possible that TXA_2 is involved in the pruritus of polycythemia vera.

Mast cells constitutively express thromboxane synthase transcripts, and the immunological stimulation of cultured mast cells produces TXA_2, the amount of which is much less than those of other arachidonic acid metabolites prostaglandin D_2 and leukotriene C_4 (Mita *et al.*, 1999). In this study, thromboxane synthase immunoreactivity was not obvious in mast cells in the normal skin. Thus, the results do not provide support for the active involvement of mast cell-derived TXA_2 in itching, but we do not deny the possibility of the involvement in itch of dermatitis.

In the skin, the TP receptor was expressed in the epidermal keratinocytes and primary afferent neurons, especially in the nerve fibers and terminals in the epidermis. TP receptors were mainly expressed in small-sized neurons in the dorsal root ganglion. Small-sized neurons have chiefly unmyelinated C fibers (Harper and Lowson, 1985: Lee *et al.*, 1986), which play an important role in itch signaling (Handwerker *et al.* (1991); Schmeltz *et al.*, 1997). U-46619 stimulated TP receptors on primary cultures of the dorsal root ganglion neurons to increase the concentration of intracellular Ca^{2+} ions. Although TP receptor is a G protein-coupled receptor (Hirata *et al.*, 1991), the action of U-44619 is inhibited by L-type calcium channel inhibitor nifedipine, suggesting that U-46619 causes membrane depolarization (Arshad *et al.*, 2006). Thus, it is suggested that TXA_2 produced by keratinocytes acts on the keratinocytes as an autocrine factor and also on primary afferents.

The increase of intracellular Ca^{2+} concentration induced by U-44619 in the primary sensory neuron subsided by 8 minutes after the application. If TP receptors on the primary afferents are primary site of pruritogenic action of U-44619, scratching elicited by intradermal U-44619 is expected to subside by 10 minutes. However, the scratching behavior peaked after 10 minutes and lasted around 50 minutes. TP receptors were also expressed on the keratinocytes, and activation by U-44619 increased the concentration of intracellular Ca^{2+} ions. Activation of keratinocytes by several kinds of stimuli releases itch mediators, for example, leukotriene B_4, nociceptin, and TXA_2 itself, and itch enhancers, for example, oxide (Andoh *et al.*, 2001, 2004; Andoh and Kuraishi (2003)). The autocrine action of TXA_2 on the keratinocytes may increase and prolong the pruritogenic action of U-44619.

Application of nucleus pulposus to the lumbar nerve root causes hyperalgesia, which is relieved by an epidural injection of a thromboxane synthase inhibitor in rats; the effect is not obvious 30 minutes after injection and becomes apparent after 3 and 7 days (Kawakami *et al.*, 2001). Thus, it is suggested that TXA_2 produced in the inflamed tissue plays a role in hyperalgesia. In this study, an intraplantar injection of U-46619 at a pruritogenic dose did not elicit pain-related response for at least 1 hour. Thus, it is suggested that TXA_2 is a cause of inflammatory hyperalgesia and a pruritogen but not an algogen.

Based on our results, the suggested mechanisms of the TXA_2-induced itching are illustrated in Figure 6. Some pruritogenic stimulation may activate the keratinocytes to produce and release TXA_2 in the epidermis. TXA_2 directly stimulates primary afferents, which conduct itch signal to the central nervous system. The action of TXA_2 on primary afferents may also result in sensitization of primary afferents to itch mediator(s) and the release of transmitter(s), for example, substance P, in the epidermis. TXA_2 and the transmitter(s) released act on the keratinocytes, which release several kinds of itch mediators and itch enhancers. At present, pruritic skin diseases in which TXA_2 is involved are not known. But thromboxane synthase and TP receptor may become new targets for the treatment of pruritic skin diseases.

MATERIALS AND METHODS

Animals

Male ICR mice (4–5 weeks old or neonatal; Shizuoka, Japan) were used. In one series of experiments, TP receptor-deficient ($TP^{-/-}$) mice with a genetic background of C57BL/6 were used at 16 weeks of age (Xiao *et al.*, 2001). The mice except neonates were housed in

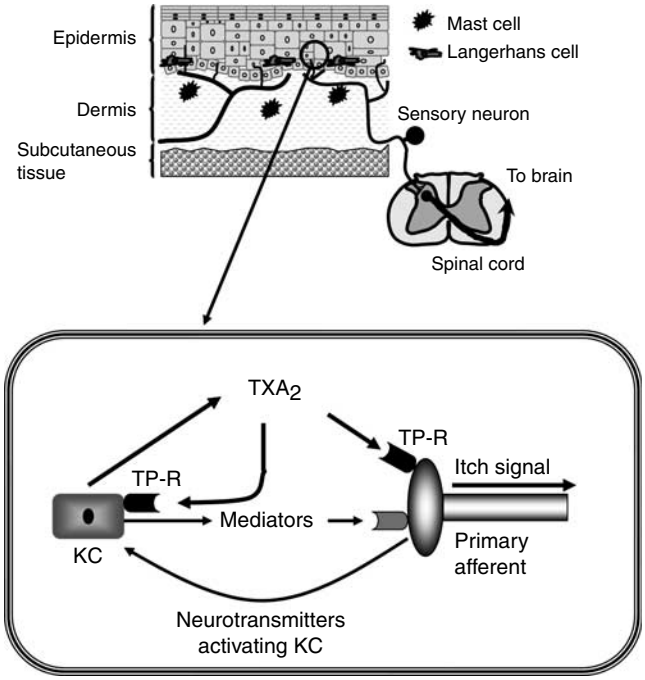

Figure 6. Schema of the mechanisms of thromboxane A_2-mediated itch. Thromboxane synthase is expressed in the epidermal keratinocytes (KC) and TP receptor (TP-R) is expressed in the KC and primary sensory neurons. KCs may produce and release thromboxane A_2 (TXA_2), which may act on primary afferents to produce itch signals. TXA_2 may also act on KCs to release itch mediators including TXA_2 itself.

a room under controlled temperature (22±1°C), humidity (55±10%), and light (lights on from 0700 to 1900 hours). Food and water were freely available. Procedures in the animal experiments were approved by the Committee for Animal Experiments at University of Toyama.

Materials

For *in vivo* experiments, 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U-46619; Cayman Chemical, Ann Arbor, MI) was dissolved in physiological saline containing 0.2 (for 1 and 10 nmol per site) or 2% ethanol (for 100 nmol per site). For *in vitro* experiment, U-46619 was dissolved in DMSO and diluted with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer. Histamine (Wako Pure Chemical Ind., Osaka, Japan) was dissolved in physiological saline. 7-[2α, 4α-(dimethylmethano)-6β-(2-cyclohexyl-2β-hydoxyacetamino)-1α-cyclohexyl]-5(Z)-heptanoic acid (ONO-3708; Ono Phamaceutical Co. Ltd., Osaka, Japan) was dissolved in saline or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer. Capsaicin (Sigma, St Louis, MI) was dissolved in 0.2% ethanol.

Behavioral experiments

For intradermal injection, the hair was clipped over the rostral part of the mouse back. The next day, U-46619 or histamine was injected intradermally in a volume of 50 μl into the interscapular region. For the observation of pain-related behavior, U-46619 was injected subcutaneously into the plantar region of the hind paw in a volume of 20 μl. Before behavior observation, the animals were placed individually into an acrylic cage composed of four cells (13 × 9 × 30 cm) for at least 1 hour for acclimation. Immediately after intradermal or subcutaneous injection, the animals were put back into the same cells and their behaviors were videotaped using an 8-mm video camera for 1 hour with personnel kept out of the observation room. Playing back of the video served for the determination of scratching of the rostral back or licking of the hind paw (Kuraishi *et al.*, 1995).

Measurement of intracellular Ca^{2+} concentration

The bilateral dorsal root ganglia at the T1–L6 levels were removed from the mice and cells were dissociated with 0.25% collagenase (Wako Pure Chemical). The cells were cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum in the presence of 2 μM cytosine arabinoside (Sigma, St Louis, MO), which kills glial cells, for at least 7 days. To prepare keratinocytes, skin was removed from neonatal mice and was treated with 0.05% collagenase A (Roche Diagnostics GmbH, Mannheim, Germany) dissolved in mouse keratinocyte growth medium (1.076% MCDB 153® (Sigma, St Louis, MI), 0.67% 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid, 0.12% $NaHCO_3$, 0.01% penicillin G, and 0.006% kanamycin) at 4°C overnight. The keratinocytes were cultured in mouse keratinocyte growth medium containing 5 μg/ml insulin, 0.5 μg/ml hydrocortisone, 14.1 μg/ml phosphorylethanolamine, 0.01 μg/ml epidermal growth factor, 10 μg/ml transferrin, and 0.1 mg/ml bovine pituitary extract. The primary cultures of dorsal root ganglion neurons and keratinocytes were washed with the medium Opti-MEM® (Invitrogen), incubated with 10 μM fluo-3/AM (Dojindo, Kumamoto, Japan) in Opti-MEM containing 0.05% poloxamer (Calbiochem, Dermstadt, Germany), and then washed with Opti-MEM. Intracellular Ca^{2+} concentration was measured using a laser-scanning microscope system (Radiance 2100; Bio-Rad, Hercules, CA) at 515–545 nm emission with excitation at 488 nm. As all of the dorsal root ganglion neurons did not express TP receptors, they were stimulated twice by U-46619 and data were obtained from neurons that responded to the second stimulation. The same procedures were applied to keratinocytes, although almost all keratinocytes responded to U-46619.

Immunocytochemistry

The dorsal root ganglia at the T1–L6 levels and the skin were removed from young mice. The dorsal root ganglion cells were prepared and cultured as mentioned above. The tissues and cultured neurons were fixed with 4% paraformaldehyde at 4°C for 1 hour and then were immersed in 30% sucrose solution overnight at 4°C. The tissues were frozen and sectioned at 30 μm with a cryostat. After being washed three times with 0.1 M phosphate buffer, the sections were treated with the first antibody at a dilution of 1/500 at 4°C overnight. Antibodies used were goat anti-TP receptor antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA), rabbit anti-thromboxane synthase antibody (Cayman Chemical) and rabbit anti-human protein gene product 9.5 antibody (Nordic Immunology, Tilburg, Netherlands). After being washed, the preparations treated with anti-TP receptor and anti-thromboxane antibodies were incubated with Alexa Fluor 488-conjugated anti-goat IgG antibody, and those treated with anti-human PGP9.5 antibody were incubated with Alexa Fluor 594-conjugated anti-rabbit IgG antibody for 1 hour at room temperature. Fluorescence signals were observed using a confocal laser scanning microscope (Bio-Rad).

Data processing

All data are presented as means ± SEM. Statistical significance was analyzed using Dunnett's multiple comparisons or Student's *t*-test; $^*P<0.05$ was considered significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We are very grateful to Ono Pharmaceutical Co. Ltd. for the gift of ONO-3708. This study was supported in part by a Grant-in-Aid for the 21st Century COE program from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

Andoh T, Kuraishi Y (1998) Intradermal leukotriene B_4, but not prostaglandin E_2, induces itch-associated responses in mice. *Eur J Pharmacol* 353:93–6

Andoh T, Kuraishi Y (2002) Inhibitory effects of azelastine on substance P-induced itch-associated response in mice. *Eur J Pharmacol* 436:235–9

Andoh T, Kuraishi Y (2003) Nitric oxide enhances substance P-induced itch-associated responses in mice. *Br J Pharmacol* 138:202–8

Andoh T, Katsube N, Maruyama M, Kuraishi Y (2001) Involvement of leukotriene B_4 in substance P-induced itch-associated response in mice. *J Invest Dermatol* 117:1621–6

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* 286:1140–5

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

Arshad M, Vijay V, Floyd BC, Marks B, Sarabu MR, Wolin MS *et al.* (2006) Thromboxane receptor stimulation suppresses gualylate cyclase-mediated relaxation of radial arteries. *Ann Thorac Surg* 81:2147–54

Daly BM, Shuster S (1986) Effect of aspirin on pruritus. *BMJ* 293:907

Felix R, Shuster S (1975) A new method for the measurement of itch and the response to treatment. *Br J Dermatol* 93:303–12

FitzGerald GA, Healy C, Daugherty J (1987) Thromboxane A_2 biosynthesis in human diseases. *Fed Proc* 46:154–8

Fjellner B, Hägermark Ö (1979) Pruritus in polycythemia vera: treatment with aspirin and possibility of platelet involvement. *Acta Dermatovener* 59:505–12

Grekas D, Alivanis P, Karamouzis M, Pyrpasopoulos M (1989) Piracetam as apotent inhibitor of plasma thromboxane B_2 during hemodialysis. *Nephron* 52:372–3

Hägermark Ö, Strandberg K (1977) Pruritogenic activity of prostaglandin E_2. *Acta Dermatovener* 57:37–43

Handwerker HO, Forster C, Kirchhoff C (1991) Dischage patterns of human C-fibers induced by itching and burning stimuli. *J Neurophysiol* 66:307–15

Harper AA, Lowson SN (1985) Conduction velocity is related to morphological cell type in rat dorsal root ganglion neurons. *J Physiol* 359:31–46

Hirata M, Hayashi Y, Ushikubi F, Yokota Y, Kagayama R, Nakanishi S *et al.* (1991) Cloning and expression of cDNA for a human thromboxane A_2 receptor. *Nature* 349:617–20

Kawakami M, Matsumoto T, Tamaki T (2001) Roles of thromboxane A_2 and leukotriene B_4 in radicular pain induced by herniated nucleus pulposus. *J Orthop Res* 19:472–7

Kroegel C, Liu MC, Hubbard WC, Lichtenstein LM, Bochner BS (1994) Blood and bronchoalveolar eosinophils in allergic subjects after segmental antigen challenge: surface phenotype, density heterogeneity, and prostanoid production. *J Allergy Clin Immunol* 93:725–34

Kuraishi Y, Nagasawa T, Hayashi K, Satoh M (1995) Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. *Eur J Pharmacol* 275:229–33

Lee KH, Chung K, Chung JM, Coggeshall RE (1986) Correlation of cell body size, axon size, and signal conduction velocity for individually labeled dorsal root ganglion cells in the cat. *J Comp Neurol* 243:335–46

Marks JG Jr, Trautlein JJ, Zwillich CW, Dermers LM (1984) Contact urticaria and airway obstruction from carbon copy paper. *JAMA* 252:1038–40

Mita H, Ishii T, Akiyama K (1999) Generation of thromboxane A_2 from highly purified human sinus mast cells after immunological stimulation. *Prostaglandins Leukot Essent Fatty Acids* 60:175–80

Mysliwiec M, Bodzenta A, Rydzewski A, Soszka J (1985) Plasama thromboxane in hemodialysis patients. *Folia Haematol Int Mag Klin Morphol Blutforsch* 112:442–6

Nagai H, Tsuji F, Inagaki N, kitagaki K, Fukutomi O, Koda A *et al.* (1991) The effects of ONO-3708, a novel TXA_2 receptor antagonist, on U-46619-induced contraction of guinea pig and human tracheal strips *in vitro* and on bronchoconstriction in guinea pigs *in vivo*. *Prostaglandins* 41:375–82

Narumiya S, Okuma M, Ushikubi F (1986) Binding of radioiodinated 13-azapinane thromboxane antagonist to platelets: correlation with antiaggregatory activity in different species. *Br J Pharmacol* 88:323–31

Narumiya S, Sugimoto Y, Ushikubi F (1999) Prostanoid receptors: structures, properties, and functions. *Physiol Rev* 79:1193–226

Needleman P, Moncada S, Bunting S, Vane JR, Hamberg M, Samuelsson B (1976) Identification of an enzyme in platelet microsomes which generates TXA_2 from prostaglandin endoperoxides. *Nature* 261:558–60

Piltch A, Sun L, Fava RA, Hayashi J (1989) Lipocortin-independent effects of dexamethasone on phospholipase activity in a thymic epithelial cell line. *Biochem J* 261:395–400

Schmeltz M, Schmidt R, Bickel A, Handwerker HO, Torebörk HE (1997) Specific C-receptors for itch in human skin. *J Neurosci* 17:8003–8

Svensson J, Hamberg M, Samuelsson B (1976) On the formation and effects of thromboxane A_2 in human platelets. *Acta Physiol Scand* 98:285–94

Veale DJ, Torley HI, Richards IM, O'Dowd A, Fitzsimons C, Belch JJ *et al.* (1994) A double-blind placebo controlled trial of Efamol Marine on skin and joint symptoms of psoriatic arthritis. *Br J Rheumatol* 33:954–8

Wahlgren C-F (1991) Itch and atopic dermatitis: clinical and experimental studies. *Acta Derm Venereol Suppl* 165:1–53

Xiao C-Y, Hara A, Yuhki K, Fujino T, Ma H, Okada Y *et al.* (2001) Roles of prostaglandin I_2 and thromboxane A_2 in cardiac ischemia-reperfusion injury: a study using mice lacking their respective receptors. *Circulation* 104:2210–5

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

Yosipovitch G, Sugeng MW, Chan TH, Goon A, Ngim S, Goh CL (2001) The effect of topically applied aspirin on localized circumscribed neurodermatitis. *J Am Acad Dermatol* 45:910–3

Zhang M-Z, Harris RC, Mckanna JA (1999) Regulation of cyclooxygenase-2 (COX-2) in rat renal cortex by adrenal glucocorticoids and mineral corticoids. *Proc Natl Acad Sci USA* 96:15280–5