Cellular/Molecular

TRPV1 Mediates Histamine-Induced Itching via the Activation of Phospholipase A_2 and 12-Lipoxygenase

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Histamine provokes itching and is a major skin disease complaint. Histamine is known to excite a subset of sensory neurons, predominantly C-fibers. Although histamine is pruritogenic, its signaling pathways that excite sensory neurons have not been identified. Because the metabolic products of lipoxygenases (LOs) activate transient receptor potential vanilloid receptor-1 (TRPV1) in sensory neurons, we hypothesized that histamine excites sensory neurons by activating TRPV1 via phospholipase A_2 (PLA_2) and LO stimulation. In cultured sensory neurons, histamine evoked inward currents that were reduced by capsazepine, a TRPV1 blocker. Moreover, histamine provoked inward currents when histamine receptor subtype 1 (H1R) and TRPV1 were expressed heterologously, but not when H1R or TRPV1 was expressed alone. In addition, histamine caused Ca^{2+} influxes in sensory neurons in wild-type mice but not in TRPV1$^{-/-}$ mice. Furthermore, histamine caused a 2.5-fold increase in the production of 12-hydroxyeicosatetraenoic acid, a metabolite of LO, in cultured sensory neurons. When injected subcutaneously into the necks of mice, histamine caused bouts of scratching, which were greatly reduced by pretreatment with capsazepine, a TRPV1 blocker, and by inhibitors of PLA_2, LO, and H1R. Furthermore, mice lacking TRPV1 markedly reduced histamine-induced scratching compared with wild type. Together, these results indicate that TRPV1 plays a key role in mediating the pruritogenic action of histamine via the PLA_2/LO pathway.

Key words: histamine; TRPV1; phospholipase A_2; 12-lipoxygenase; itch; pruritus

Introduction

Itching (pruritus) is a sensation felt on an area of skin that provokes scratching. Itching is stressful and reduces the quality of life when excessive or chronic. People with chronic pruritus cannot lead a normal life because of the psychological disturbances associated with depression or sleep deprivation (Sheehan-Dare et al., 1990; Hashiro and Okumura, 1997). Approximately 10% of children worldwide suffer from atopic dermatitis (Flohr et al., 2004) accompanying severe pruritus. A number of substances are known to cause pruritus; the most well known of which is histamine (Stander et al., 2003). Although the role of histamine in the itching associated with dermatitic skin is still unclear, it is unequivocal that histamine induces severe itching when it is applied to skin experimentally (Magerl et al., 1990; Schmelz et al., 1997, 2003).

Histamine is known to be released from mast cells when tissues are inflamed or stimulated by allergens (Benditt et al., 1955; Rowley and Benditt, 1956), and it excites a subset of unmyelinated C-fibers (Tani et al., 1990). Among these C-fibers, histamine preferentially excites mechano-insensitive primary afferent fibers (Schmelz et al., 1997, 2003). In addition, itching fibers are mostly conveyed to spinothalamic tract neurons in the superficial lamina of the spinal cord, which represents specific conduction pathways (Andrew and Craig, 2001). Although the pathophysiological role of histamine in pruritus has been rigorously studied, the precise downstream signaling pathway required for the excitatory action on sensory neurons is not understood.

Transient receptor potential vanilloid receptor-1 (TRPV1) is a nonselective cation channel activated by capsaicin, heat, and acid (Caterina et al., 1997). Because TRPV1 is activated by pain-causing stimuli, its role in nociception has been predicted. Indeed, TRPV1-deficient mice show impaired responses to some forms of noxious thermal stimuli (Caterina et al., 2000; Davis et al., 2000). Many forms of endogenous lipids activate TRPV1. For example, endocannabinoids such as anandamide and N-arachidonoyl dopamine activate TRPV1 (Zygmunt et al., 1999; De Petrocellis et al., 2004). In addition, TRPV1 is activated by metabolic products of lipoxygenase (LO), most notably by 12-hydroperoxyeicosatetraenoic acid (12-HPETE), which has a three-dimensional structure similar to that of capsaicin (Hwang et al., 2000). Furthermore, upstream signals that activate TRPV1 via the production of 12-HPETE have been postulated (Shin et al., 2002). Bradykinin, a pain-causing substance that is released from inflammatory tissues, is also known to activate TRPV1 using PLA_2 and LO pathway in sensory neurons (Shin et al., 2002).

H1R is a member of the G-protein-coupled receptor family and is known to couple with $G_{q/11}$ (Gutowski et al., 1991). Stimulation of H1R leads to the accumulation of arachidonic acid via the activation of PLA_2 (Leurs et al., 1994). Because LO metabo-

Received Oct. 26, 2006; revised Jan. 5, 2007; accepted Jan. 5, 2007.

This work was supported by the National Creative Research Initiative of the Korean Ministry of Science and Technology.

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DOI:10.1523/JNEUROSCI.4643-06.2007

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lizes arachidonic acid to various eicosanoids, including 12-HPETE, it is likely that H1R activation leads to the activation of TRPV1 in sensory neurons. Therefore, in this study, we sought to determine whether histamine activates TRPV1 via the stimulations of PLA_2 and LO to excite sensory neurons.

Materials and Methods

Primary culture of dorsal root ganglion neurons. Dorsal root ganglion (DRG) neurons were primarily cultured for electrophysiology and confocal microscopy as described previously (Shin et al., 2002). Briefly, thoracic and lumbar DRGs were dissected and collected from neonatal rats or adult mice. The culture medium, a mixture of DMEM and Ham's F-12 solution (50:50), contained 10% fetal bovine serum, 1 mM sodium pyruvate, 50–100 ng/ml nerve growth factor (Invitrogen, Gaithersburg, MD), and 100 U/ml penicillin/streptomycin. Ganglia were incubated for 30 min at 37°C with 1 mg/ml collagenase (Worthington Biochemical, Freehold, NJ) followed by 30 min at 37°C with 2.5 mg/ml trypsin (BD Biosciences, Indianapolis, IN). Dissociated cells were plated on poly-L-lysine-treated small coverslips and incubated for 2 or more days at 37°C in 95% air/5% CO_2.

Cloning of rat H1R and rat TRPV1. First-strand cDNA was reverse transcribed from total RNA isolated from a rat DRG neuron using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) and oligo dT primers. Full-length coding sequence regions of rat histamine receptor type 1 (Hrh1) (NM_017018) and rat TRPV1 (NM_031982) were obtained by reverse transcription-PCR using custom-synthesized oligomers. Genes were inserted into pXOON and pcDNA3.1 (Invitrogen) expression vector, respectively. pXOON (Jespersen et al., 2002), an expression vector containing an enhanced green fluorescent protein marker, was a gift from Dr. Thomas Jespersen (University of Copenhagen, Copenhagen, Denmark). All genes were sequenced and showed 100% identity with GenBank sequences.

Electrophysiology. Hrh1–pXOON and TRPV1–pcDNA3.1 were cotransfected into HEK293T cells using a LipofectAMINE PLUS kit (Invitrogen). Two or 3 d after the transfection, whole-cell currents were recorded from cultured human embryonic kidney (HEK) 293T cells. Whole-cell currents were also recorded from cultured DRG neurons isolated from a 1-d-old neonatal rat. When a borosilicate glass pipette (World Precision Instruments, Sarasota, FL) coated with Sylgard (Dow Corning, Midland, MI) touched the surface of a cell, gentle suction was applied to the pipette to form a gigaseal. Subsequently, the membrane in contact with the pipette was ruptured by applying suction to form a whole cell. After a whole cell had been formed, capacitive transients were canceled. The pipette solution contained the following (in mM): 4 ATP, 0.1 GTP, 130 KCl, 2 $MgCl_2$, 5 EGTA, and 10 KOH/HEPES, pH 7.2. The bath solution contained the following (in mM): 130 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, and 10 NaOH/HEPES, pH 7.2. Experiments were performed at room temperature. All data were recorded using Digidata 1440A and pClamp 10 software (Molecular Devices, Sunnyvale, CA).

12(S)-hydroeicosatetraenoic acid immunoassay. To measure the level of 12-HPETE, an endogenous activator of TRPV1, an amount of 12(*S*)-hydroeicosatetraenoic acid (12-HETE), which is an immediate metabolic product of 12-HPETE, was measured instead because of the unstable nature of 12-HPETE (Hwang et al., 2000). Primary cultures of DRGs isolated from wild-type C57BL/6J or TRPV1 $^{-/-}$ mice were used to measure levels of 12-HETE productions as described previously (González-Núñez et al., 2001; Shin et al., 2002). Trace amounts of 12-HETE possibly released from cultured DRG cells were determined by enzyme immunoassays using specific polyclonal antibody to 12-HETE. 12-HETE antibody shows low cross-reactivity with other eicosanoid compounds, such as 15-HETE (0.3%) and 12(*R*)-HETE (2.5%). Because not all 12-HPETE is metabolized into 12-HETE, the reducing agent, L-cysteine (1 mM), was added for 3 min after histamine application to induce a complete reduction of 12-HPETE to 12-HETE (Chang et al., 1982). Primary cultures of DRG cells were incubated with 100 μM histamine for 5 min. These samples and standards were plated in 96-well plates coated with goat anti-rabbit IgG. 12-HETE enzyme immunoassay conjugate and antibody were added to each well and incubated for 2 h, and each well was then emptied and washed three times. After adding *p*-nitrophenyl phosphate solution as a substrate, plates were incubated for 3 h at 37°C. The reaction was stopped with trisodium phosphate solution, and optical densities were immediately read at 405 nm (Spectrafluor microplate reader; Tecan, Salzburg, Austria). 12-HETE concentrations were determined using standard curves.

Measurement of intracellular Ca^{2+} concentration. Cytosolic free Ca^{2+} ion concentrations were measured using a confocal laser-scanning microscope (Leica, Heidelberg, Germany). Cultured DRG cells isolated from wild-type C57BL/6J or TRPV1 $^{-/-}$ mice were loaded with 5 μM Fluo-3/AM (Invitrogen, Eugene, OR) and incubated for 1 h at 37°C. Fluo-3/AM was excited at 488 nm, and emitted fluorescence was measured at 515 nm. Intracellular Ca^{2+} changes are expressed as F/F_0 ratios, where F_0 is the initial fluorescence intensity.

Measurements of mRNA levels. First-strand cDNA was reverse transcribed from total RNA isolated from both wild-type and TRPV1 $^{-/-}$ DRG neurons using the SuperScript III First-Strand Synthesis System (Invitrogen). Custom oligomers that were designed to amplify genes of interest produced PCR products in the range 150 ~ 250 bp, except for TRPV1. All PCR experiments were performed over 35 cycles of 94°C (30 s), 60°C (30 s), and 72°C (30 s) using ExTaq (Takara, Kyoto, Japan). The primers used were as follows: mouse H1R (1050–1202), 5′-GACCTTGGTGGATCGACAGT-3′ (forward) and 5′-TGTCTGGAATGTGAGCGAAG-3′ (reverse); mouse TRPV1 (327–2504), 5′-GAGGCTCTATGATCGCAGGA-3′ (forward) and 5′-GCCATGGAATCCTTGAAGAC-3′ (reverse); mouse 12-LO (1155–1375), 5′-CTTCAAGCTCCTCGTTCCAC-3′ (forward) and 5′-GAGCACTTGGGATTCTCAGC-3′ (reverse); mouse $cPLA_2$ (412–650), 5′-GTTTGTTCATGCCCAGACCT-3′ (forward) and 5′-ATCCCCGACTCATACAGTGC-3′ (reverse); mouse GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (493–715), 5′-AACTTTGGCATTGTGGAAGG-3′ (forward) and 5′-ACACATTGGGGGTAGGAACA-3′ (reverse).

Scratch test. Wild-type Institute of Cancer Research (ICR) mice and C57BL/6J mice (7 weeks of age) were purchased from Orient Laboratory Animals (Seoul, Korea). TRPV1 $^{-/-}$ mice of C57BL/6J strain were purchased from The Jackson laboratory (Bar Harbor, ME). To measure itch-related behavior induced by histamine, 2 ~ 500 μM histamine in 200 μl (0.4 ~ 100 nmol) was injected into the neck intradermally. After histamine injection, bouts of scratching were counted for 20 min. Blockers or inhibitors were given intraperitoneally (200 μl) 30 min before the histamine challenge.

Statistics. All data were presented as means ± SEM. One-way ANOVA with Tukey's *post hoc* test was used to compare multiple means.

Results

Histamine evokes an inward current in primary cultured DRG neurons

When 100 μM histamine was applied to primary cultures of ICR rat neonatal DRG neurons, small inward currents were activated in a small proportion of neurons tested (12 of 70 cells, 17.1%) with an average amplitude of 134.1 ± 10.7 pA (Fig. 1*A*). These histamine-sensitive currents were reduced by a TRPV1 antagonist, capsazepine (10 μM; $n = 6$), suggesting that histamine activates TRPV1 (Fig. 1*A*). The proportion of neurons that responded to histamine was comparable with the proportion (8 of 56 U, 14.3%) of histamine-responding fibers among cutaneous fibers tested (Schmelz et al., 1997).

Histamine evokes an inward current in HEK 293T cells coexpressing H1R and TRPV1

Because histamine-induced response was small and infrequent, it was difficult to perform pharmacological experiments in cultured sensory neurons. Therefore, we attempted to reconstitute histamine-evoked responses in a heterologous expression system. To do this, rat H1R alone or with rat TRPV1 cDNA was transfected into HEK 293T cells. When H1R was transfected alone, the application of histamine (10 μM) failed to evoke inward currents

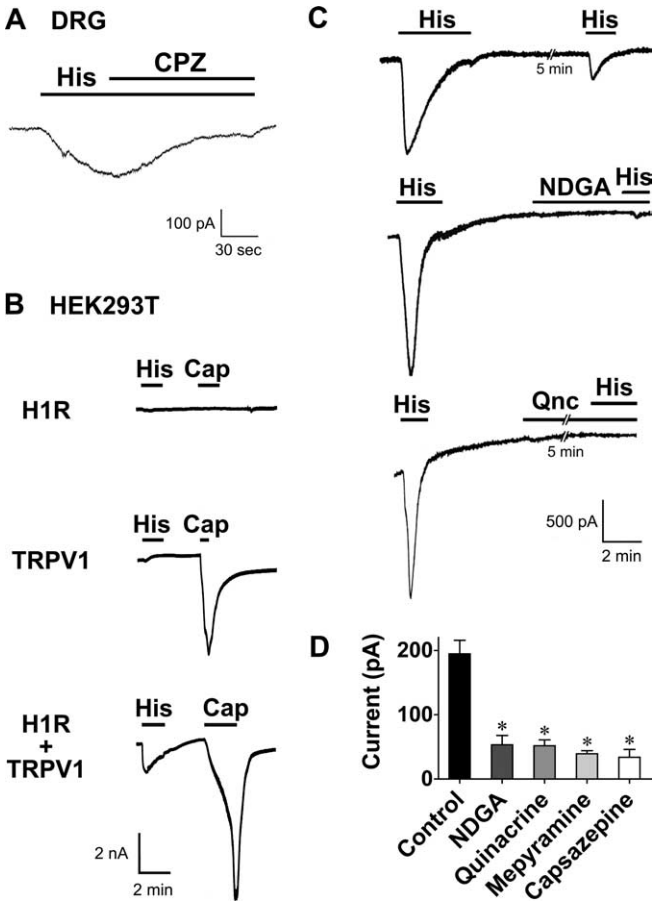

Figure 1. Histamine induced currents in DRG neurons and in HEK 293T cells transfected with TRPV1 and H1R that were blocked by inhibitors or blockers of PLA_2, LO, TRPV1, and H1R. ***A***, In rat neonatal DRG neurons, application of 100 μM histamine (His) evoked an inward current that was blocked by treatment with a TRPV1 antagonist, capsazepine (CPZ; 10 μM). Holding potential was −60 mV. ***B***, HEK293T cells were transfected with either H1R or TRPV1 alone or transfected with H1R and TRPV1. A current response to 10 μM histamine was observed only when cells were transfected with both H1R and TRPV1 (bottom). Application of 1 μM capsaicin (Cap) evoked inward currents when cells were transfected with TRPV1 alone (middle) or with H1R (bottom). ***C***, Trace examples of the effects of inhibitors of PLA_2 [quinacrine (Qnc)] or LO (NDGA) on histamine-induced currents in HEK cells transfected with H1R and TRPV1. In HEK cells expressing H1R and TRPV1, a second histamine challenge induced a clear tachyphylaxis. Pretreatment with NDGA or quinacrine remarkably abolished this second histamine-evoked influx (middle and bottom). ***D***, Summary of effects of NDGA ($n = 9$), quinacrine ($n = 9$), mepyramine (H1R antagonist; $n = 7$), and capsazepine ($n = 7$) on current responses to a second histamine challenge. Error bars indicate SEMs. $^*p < 0.001$.

($n = 7$) (Fig. 1*B*). Likewise, cells transfected with TRPV1 alone failed to show currents in response to histamine application but showed inward currents in response to the application of 1 μM capsaicin ($n = 6$) (Fig. 1*B*). In contrast, when transfected with both H1R and TRPV1, 10 μM histamine evoked robust inward currents in all cells tested ($n = 82$) (Fig. 1*B*,*C*). Application of 1 μM capsaicin also exhibited a robust inward current in H1R/TRPV1-transfected cells. The magnitude of histamine-evoked current was much smaller than that induced by capsaicin (Fig. 1*B*). Together, these results suggest that histamine activates TRPV1.

Because histamine response desensitized rapidly, it was difficult to test the effects of various inhibitors or blockers. Therefore, we applied histamine twice to examine these effects. Blockers or inhibitors of PLA_2, LO, or TRPV1 were applied before and during the second histamine challenge. A clear tachyphylaxis occurred as the second histamine application elicited much smaller current responses, reaching an amplitude of only 28.4 ± 3.1% that of the first histamine challenge (759.0 ± 128.6 vs 194.6 ± 21.1 pA; $n = 9$) in H1R/TRPV1-expressing cells (Fig. 1*C*). In H1R/TRPV1-expressing cells, the TRPV1 antagonist capsazepine (10 μM) significantly reduced current response to this second histamine challenge (44.4 ± 11.0 pA; $n = 7$; $p < 0.001$). Likewise, pretreatment with 10 μM mepyramine, a specific blocker for H1R, also blocked histamine-induced currents (39.3 ± 4.8 pA; $n = 6$; $p < 0.001$). Similarly, pretreatments with 10 μM nordihydroguaiaretic acid (NDGA), a nonspecific LO inhibitor, or quinacrine (10 μM), a PLA_2 inhibitor, significantly reduced the histamine-induced currents by 53.2 ± 14.5 pA ($n = 9$; $p < 0.001$) and 51.8 ± 9.1 pA ($n = 7$; $p < 0.001$), respectively (Fig. 1*C*,*D*). Thus, these results indicate that histamine activates TRPV1 via the stimulations of PLA_2 and LO.

Calcium influx in the DRG neurons of TRPV1 $^{-/-}$ mice

To identify the correlation between histamine and TRPV1, calcium-imaging techniques were used on primary cultured DRG neurons isolated from C57BL/6J wild-type and TRPV1-deficient mice. The application of histamine (100 μM) to DRG neurons of wild-type mice evoked rapid increases in intracellular Ca^{2+} in 62.7% of the cells tested (47 of 75) (Fig. 2*A*). Among these 47 histamine-sensitive cells, 35 cells (66.0%) also responded to 10 μM capsaicin (data not shown). Moreover, this histamine- and capsaicin-induced Ca^{2+} influx was not observed when Ca^{2+}-free solution was applied to baths (data not shown). In contrast, 100 μM histamine failed to induce appreciable Ca^{2+} influx in the majority of cells (70 of 80 cells) isolated from TRPV1-deficient mice. In 10 of 80 sensory neurons from TRPV1 $^{-/-}$ mice, histamine application induced a small increase in intracellular Ca^{2+} (data not shown). Furthermore, the application of 10 μM capsaicin failed to induce an increase in intracellular Ca^{2+} in the DRG neurons of TRPV1 $^{-/-}$ mice (data not shown).

We then checked the mRNA levels of H1R, PLA_2, LO, and TRPV1 in sensory neurons from wild-type and TRPV1 $^{-/-}$ C57BL/6J mice using reverse transcriptase-PCR. As shown in Figure 2*D*, the mRNA levels of H1R, PLA_2, and LO in TRPV1 $^{-/-}$ mice were similar to those in wild-type mice. However, TRPV1 mRNA was absent in sensory neurons isolated from TRPV1 $^{-/-}$ mice. Thus, the loss of histamine response in TRPV1 $^{-/-}$ mice is attributable to the loss of TRPV1 and not to other factors in the histamine signaling pathway. These results further suggest that histamine causes Ca^{2+} influx via the activations of PLA_2, LO, and TRPV1.

Production of 12-HETE by histamine in sensory neurons

The activation of TRPV1 by histamine is presumed to be mediated by the production of 12-HPETE. Thus, it appeared necessary to check whether histamine increases the production of 12-LO metabolites in sensory neurons. To address this issue and because of instability of 12-HPETE, we measured the level of 12-HETE instead in cultured DRG cells isolated from a C57BL/6J mouse, which is an immediate metabolite of 12-HPETE. 12-HETE levels were measured using enzyme immunoassays with specific polyclonal antibody to 12-HETE.

The application of 10 μM histamine induced about a 2.5-fold increase in the production of 12-HETE in DRG cells isolated from wild-type C57BL/6J mice (1.87 ± 0.19 vs 4.72 ± 0.46 pg/10^3 cells; $n = 6$; $p < 0.001$). Moreover, histamine-induced increases in 12-HETE production were inhibited by the coapplication of 10

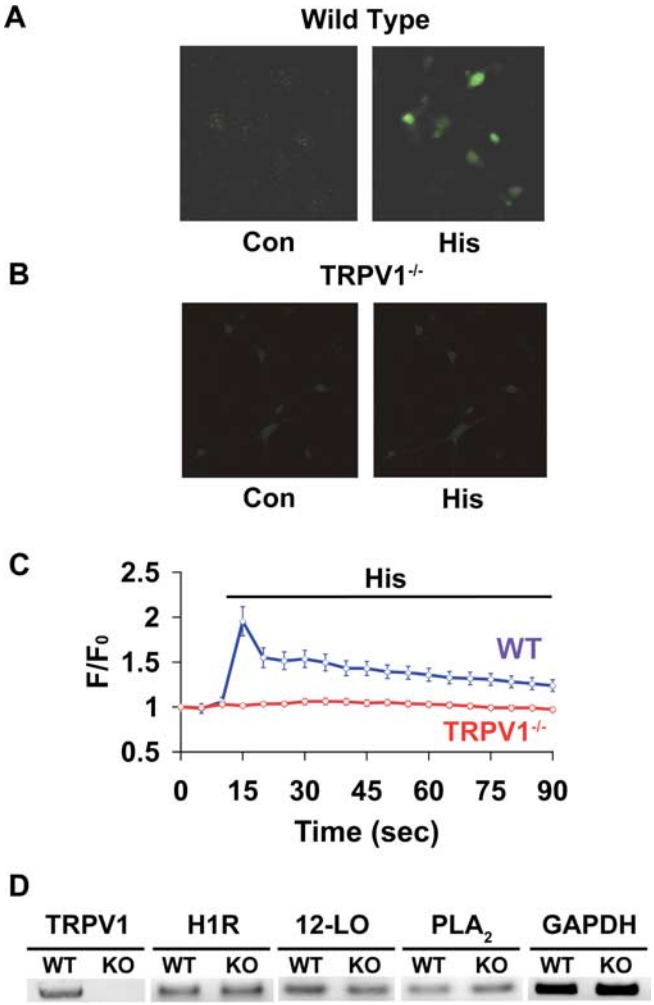

Figure 2. ***A***, ***B***, Histamine (His; 100 μM) challenge elicited increased intracellular Ca^{2+} in DRG neurons of wild-type mice (***A***) but not in DRG neurons of TRPV1 $^{-/-}$ mice (***B***). ***C***, Summary of Ca^{2+} influx induced by histamine application in cultured sensory neurons isolated from wild-type (WT; $n = 75$) or TRPV1 $^{-/-}$ ($n = 80$) mice. F_0 denotes initial fluorescence detected at time 0. ***D***, mRNA levels of H1R, cytosolic PLA_2 (PLA_2), 12-LO, and TRPV1 in DRG cells isolated from TRPV1 $^{-/-}$ mice. Notice that no significant differences in mRNA levels were found except for TRPV1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control. Error bars indicate SEMs. Con, Control.

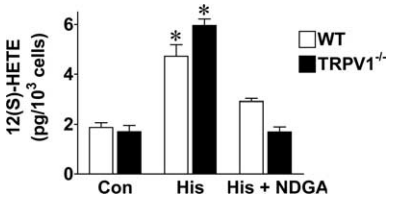

Figure 3. Application of histamine (His) increased the levels of 12(*S*)-HETE in primary cultured DRG cells from wild-type (WT) C57BL/6J as well as TRPV1 $^{-/-}$ (filled bars) mice, as determined by immunoassay. Noticeable increase in 12(*S*)-HETE levels were observed after 10 μM histamine application versus nontreated controls in DRG cells of both types of mice ($n = 6$). This increase was blocked when 10 μM NDGA was cotreated with histamine ($n = 6$). Error bars indicate SEMs. Con, Control.

μM NDGA, an LO inhibitor (2.91 ± 0.12 pg/10^3 cells; $n = 6$) (Fig. 3). Increases in 12-HETE levels by histamine were also observed in TRPV1-deficient C57BL/6J mice. In the sensory neurons of TRPV1 $^{-/-}$ mice, the level of 12-HETE was increased to 5.96 ± 0.26 pg/10^3 cells ($n = 6$) from 1.70 ± 0.25 pg/10^3 cells ($n = 6$)

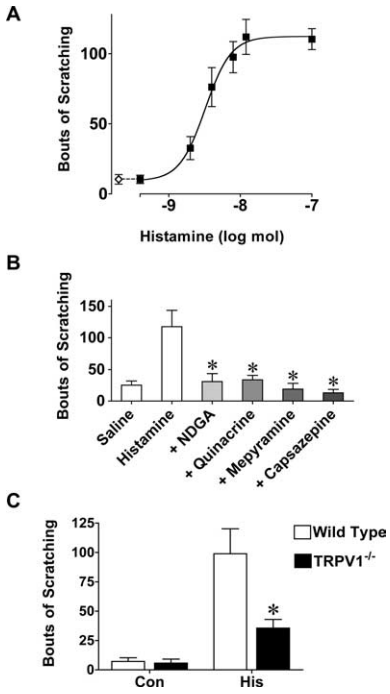

Figure 4. Intradermal histamine injection in mice induced scratching, which is attenuated when factors in histamine signaling pathway are hampered. ***A***, Dose–response curve of histamine-induced scratching in ICR mouse. Histamine (0.4 ~ 100 nmol in 200 μl; filled squares; $n = 5 \sim 9$) or saline (open diamond; $n = 8$) was injected to the dorsum of the neck intradermally of ICR mice. Bouts of scratching with hind limbs were counted for 20 min. ***B***, Effects of blockers or inhibitors of the proposed histamine signaling pathway on histamine-induced scratching. Various blockers or inhibitors were first treated intraperitoneally 30 min before the histamine (8 nmol) injection ($n = 7 \sim 8$). ***C***, Intradermal histamine (His) induced scratching in wild-type C57BL/6J ($n = 8$) or TRPV1 $^{-/-}$ mice ($n = 12$) compared with saline injections ($n = 5$). Notice that TRPV1 $^{-/-}$ mice showed reduced histamine-induced scratching compared with the wild-type controls. *$p < 0.001$. Error bars indicate SEMs. Con, Control.

when histamine was applied. This histamine-induced increase in 12-HETE was also completely blocked by 10 μM NDGA (1.69 ± 0.20 pg/10^3 cells; $n = 6$) (Fig. 3). These results indicate that histamine evokes the production of 12-HETE in sensory neurons via LO activation and that histamine also induces the production of 12-HETE even in TRPV1-deficient mice. These results also demonstrate that the lack of histamine response in TRPV1-deficient mice is attributable to the lack of TRPV1 expression and not to other factors in the histamine-signaling pathway.

Histamine induced scratching in wild-type but not in TRPV1 $^{-/-}$ mice

Because histamine is known to provoke itching, we determined whether the histamine signaling pathway is relevant *in vivo*. Because intradermal histamine is known to induce neck scratching (Bell et al., 2004), bouts of scratching were counted for 20 min after histamine of 0.4 ~ 100 nmol was injected intradermally. Intradermal injection of histamine >4 nmol evoked robust scratching behavior in a dose-dependent manner (Fig. 4*A*). When 4 nmol of histamine was injected intradermally, 76.2 ± 13.9 ($n = 5$) bouts of scratching were observed, more frequent than observed with saline-injected control ICR mice (10.4 ± 3.4 bouts; $n = 8$). The apparent ED_{50} of histamine in evoking scratching was 3.23 nmol, which was comparable with those observed by others (Maekawa et al., 2000; Inagaki et al., 2001). We did not observe flinching or vocalization that was a typical indication of nociceptive or nocifensive reflexes (Bell et al., 2004)

even after the maximal dose of histamine injection. Furthermore, for a positive control for itch-related behavior, 100 nmol serotonin that is known to evoke itching in mice (Yamaguchi et al., 1999) was injected intradermally. The intradermal injection of 100 nmol of serotonin also caused scratching (210.6 ± 32.2 bouts; $n = 5$). We then performed pharmacological intervention if inhibitors of PLA_2, LO, and TRPV1 block the histamine-induced scratching. In control ICR mice, a histamine (8 nmol) injection caused 117.7 ± 26.0 ($n = 7$) bouts of scratching in 20 min, whereas intradermal saline caused only 25.3 ± 6.7 ($n = 8$) bouts (Fig. 4*B*). In contrast, a marked reduction in histamine-induced scratching bouts (13.0 ± 5.6 bouts; $n = 8$; $p < 0.001$) was observed when capsazepine (4 mg/kg) was given intraperitoneally 30 min before the histamine challenge. Similarly, significant reductions in scratching were observed when NDGA (50 mg/kg), quinacrine (20 mg/kg), or mepyramine (40 mg/kg) were given intraperitoneally before histamine challenge (Fig. 4*B*). Histamine-induced scratching behavior was also determined in TRPV1 $^{-/-}$ C57BL/6J mice. As shown in Figure 4*C*, when histamine (8 nmol) was injected intradermally, scratching behavior was markedly less frequent in TRPV1 $^{-/-}$ mice (35.6 ± 7.3 bouts of scratches; $n = 12$; $p < 0.01$) than in the wild-type C57BL/6J mice (98.9 ± 21.3 bouts of scratches; $n = 8$). Intradermal injection of saline caused only 7.2 ± 3.2 ($n = 5$) and 5.8 ± 3.3 ($n = 5$) bouts of scratches in wild-type and TRPV1 $^{-/-}$ C57BL/6J mice, respectively (Fig. 4*C*). In contrast, intradermal injection of serotonin (100 nmol), another itch-causing substance (Yamaguchi et al., 1999), induced an equal number of scratches in wild-type (103.1 ± 12.7; $n = 10$) as well as TRPV1-deficient C57BL/6J mice (85.7 ± 22.4; $n = 6$; $p = 0.47$). Together, these results clearly suggest that TRPV1 mediates histamine-induced itching via the activation of the PLA_2/LO pathway *in vivo*.

Discussion

Histamine has been considered to be a main itch-inducing substance for many years. Antihistamines, which block histamine receptors, are commonly used to treat pruritus, although sometimes they are ineffective (Wallengren, 2005). More direct evidence for the cause of pruritus induced by histamine is provided by the fact that the cutaneous application of histamine induces itching in man (Heyer et al., 1989, 1997). Animals injected with histamine also show typical scratching behavior (Laidlaw et al., 2002). However, although the biochemical pathways for its synthesis and the pathophysiological role of histamine are well documented, little is known of the signaling pathway that leads to the excitation of the sensory neurons, which generates the appropriate neural signals for itching. The present study provides *in vivo* and *in vitro* evidence that histamine requires the activation of TRPV1 to excite these sensory neurons. Histamine was found to evoke inward currents and Ca^{2+} influx that were dependent on the activities of TRPV1, PLA_2, and LO with concomitant 12-HETE elevation in sensory neurons. Most notably, histamine induced scratching in a PLA_2-, LO-, and TRPV1-dependent manner in wild-type mice but not in TRPV1-deficient mice.

A possible link between histamine and TRPV1 in sensory neurons has been suggested in the literature. TRPV1 and histamine receptors are expressed in a subset of sensory neurons (Nicolson et al., 2002; Taylor-Clark et al., 2005), and primary afferent C-fibers that respond to histamine are also sensitive to capsaicin (Schmelz et al., 1997; Nicolson et al., 2002). Moreover, cutaneous capsaicin application often evokes itching as well as painful sensation (Green, 1990; Green and Shaffer, 1993). Furthermore, repeated application of capsaicin is known to desensitize TRPV1 or sensory nerves and was found to alleviate the pruritus induced by histamine (Handwerker et al., 1987). Together, these results further solidify the notion that TRPV1 mediates histamine-induced itching.

Because TRPV1 is present in nociceptors causing some type of pain (Caterina et al., 1997; Caterina et al., 2000), it would be puzzling to accept how TRPV1 mediates nociception as well as itch if it is present in pruritic and algesic primary afferent fibers. Obviously, it is not the type of channels that determines the type of sensations. Instead, it is the type of primary afferent fibers that determines pain or itch. Because itch fibers and nociceptors are functionally segregated (Schmelz et al., 1997; Andrew and Craig, 2001), activation of TRPV1 excites either pruritic or algesic primary afferent fibers, which leads to itch or pain sensations. Thus, nociception and itch sensation likely depend on the type of primary afferent fibers that are excited by the activation of TRPV1. In this regard, capsaicin can cause both itch and pain because it activates TRPV1 in both types of primary afferent fibers. Indeed, although predominant sensation evoked by capsaicin is pain, topical application of capsaicin to the skin also causes itch as well as pain (Green and Flammer, 1989; Green, 1990; Green and Shaffer, 1993). Our data precisely explain why capsaicin evokes itch as well as pain. It is also noteworthy that noxious stimulation such as scratching, heat, cold, and capsaicin suppresses or relieves itch (Bickford, 1937; Ward et al., 1996; Weisshaar et al., 1998; Brull, 1999; Yosipovitch et al., 2005). Although the precise mechanisms are not known, it is generally accepted that nociceptive signals inhibit incoming itch signals by central mechanism (Bickford, 1937; Brull et al., 1999; Yosipovitch et al., 2005). Therefore, this central inhibitory effect of nociceptive signals on itch input in part explains why capsaicin evokes pain predominantly although it is highly likely to excite both nociceptors and pruritic sensory fibers.

Because TRPV1 is a thermal sensor that detects noxious heat (Caterina et al., 1997), it is highly likely that change in temperature affects itch sensation. It is commonly experienced as well as tested experimentally that warming aggravates itch (Mizumura and Koda, 1999), whereas itch is suppressed by noxious heat. The most probable explanation for aggravation of itch by warming would be that TRPV1 is sensitized by inflammatory factors in pathological condition in skin such as dermatitis, which possibly lowers the heat threshold (Sugiura et al., 2002; Moriyama et al., 2005) and increases the sensitivity to endogenous ligands in TRPV1 activation. Thus, when the affected areas are warmed, it causes more intense itch. Warming also would facilitate enzymatic reactions mediated by PLA_2 or lipoxygenase in the histamine signaling pathway, resulting in production of more lipoxygenases products, causing intense itch signals. In contrast, noxious heat is known to suppress itch, possibly via central mechanisms, as explained above (Yosipovitch et al., 2005).

Leukotriene B_4 is a metabolic product of 5-lipoxygenase (Borgeat and Samuelsson, 1979a,b). Like 12-HPETE, leukotriene B_4 is capable of activating TRPV1 (Hwang et al., 2000). Interestingly, leukotriene B_4 is abundantly expressed in the skins of atopic dermatitis patients (Ruzicka et al., 1986; Fogh et al., 1989). The application of leukotriene B_4 to skin induces itching, whereas prostaglandin E_2, another arachidonic acid metabolite synthesized by cycloxygenase, does not (Andoh and Kuraishi, 1998). The induction of itching by leukotriene B_4 is thought to be mediated primarily by its receptor in sensory neurons, because blockers of leukotriene B_4 receptors alleviate the itching of dermatitic skin or leukotriene B_4-induced scratches (Matsui et al.,

1994; Kanai et al., 1995). However, some proportion of the itching induced by leukotriene B_4 is attributable to receptor-independent mechanisms in sensory neurons (Andoh and Kuraishi, 1998), which may include the direct action of leukotriene B_4 on TRPV1 in sensory neurons because of its agonistic activity on TRPV1 (Hwang et al., 2000). Thus, these clinical and experimental data further support the idea that LO products excite sensory neurons via the activation of TRPV1 as we propose for the histamine signaling pathway.

Two major intracellular signaling pathways are known to mediate H1R responses in various cells. Histamine increases intracellular Ca^{2+} and concomitantly increases the levels of inositol phosphates in Chinese hamster ovarian cells transfected with H1R (Leurs et al., 1994). The increases in intracellular Ca^{2+} induced by H1R stimulation are mostly dependent on intracellular Ca^{2+} stores because increases in intracellular Ca^{2+} remain intact in Ca^{2+}-free medium (Leurs et al., 1994). Moreover, release from intracellular Ca^{2+} stores is thought to be mediated by phospholipase C/inositol 1,4,5-trisphosphate pathway (Leurs et al., 1994). However, increases in intracellular Ca^{2+} from intracellular Ca^{2+} storage after histamine treatment in the heterologous HEK cells sharply contrasts with the origin of increase in intracellular Ca^{2+} in sensory neurons. In sensory neurons, increases in intracellular Ca^{2+} originate from extracellular Ca^{2+} because increases in intracellular Ca^{2+} by histamine are completely absent in Ca^{2+}-free medium (Kim et al., 2004). Moreover, in sensory neurons, influx through TRPV1 is responsible for increases in intracellular Ca^{2+} because such increases are not observed in sensory neurons isolated from TRPV1-deficient mice (Fig. 2). Furthermore, histamine-induced intracellular Ca^{2+} or currents are blocked by TRPV1 antagonist (Fig. 1). The production of arachidonic acid (mainly by PLA_2) is another intracellular signal stimulated by H1R (Leurs et al., 1994). In the present study, a PLA_2 inhibitor blocked histamine-induced currents and scratching behavior. In addition, the production of 12-HETE, a downstream metabolite of PLA_2, was augmented when histamine was applied to sensory neurons (Fig. 3). Therefore, of the two proposed pathways, namely phospholipase C/inositol 1,4,5-trisphosphate and PLA_2/LO, histamine prefers the latter for sensory neuron excitation.

Although the results of the present study suggest that histamine excites sensory neurons via the PLA_2/LO/TRPV1 pathway, we cannot rule out the possibility that other parallel pathways activate TRPV1 by histamine. In the present study, blockers and inhibitors of PLA_2, LO, or TRPV1 failed to completely block histamine response. Instead, a proportion of histamine response remained after inhibitor application. As was found for phosphoinositide increase by histamine, histamine may stimulate the phospholipase C/protein kinase C signal to eventually sensitize TRPV1 in sensory neurons (Premkumar and Ahern, 2000). In addition, phospholipase C sensitizes TRPV1 indirectly by sequestering phosphatidylinositol 4,5-bisphosphate that constitutively inhibits TRPV1 (Prescott and Julius, 2003). Therefore, signaling pathways other than the PLA_2/LO/TRPV1 pathway are also likely to mediate histamine response in sensory neurons.

In summary, the present study demonstrates that histamine excites sensory neurons by activating TRPV1 and that this activation is mediated by the production of 12-HPETE, a downstream metabolite of PLA_2 and LO. The proposed PLA_2/LO/TRPV1 pathway accounts for the scratching evoked by histamine. Moreover, because histamine is a major cause of itching in dermatitis patients, the present study provides clues concerning the treatment of itching-related conditions and inflammatory pain (Oh, 2006).

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