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Histamine-induced Ca^{2+} influx via the PLA_2/lipoxygenase/TRPV1 pathway in rat sensory neurons☆

Byung Moon Kim, Sang Hee Lee, Won Sik Shim, Uhtaek Oh*

Sensory Research Center, National Creative Research Initiatives, College of Pharmacy, Seoul National University, San 56-1, Shinlim, Kwanak-Gu, Seoul 151-742, South Korea

Abstract

Histamine is known to excite a subset of C-fibers and cause itch sensation. Despite its well-defined excitatory action on sensory neurons, intracellular signaling mechanisms are not understood. Previously, we demonstrated that bradykinin excited sensory neurons by activating TRPV1 via the phospholipase A_2 (PLA_2) and lipoxygenase (LO) pathway. We, thus, hypothesized that histamine excited sensory neurons via the PLA_2/LO/TRPV1 pathway. Application of histamine elicited a rapid increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) that desensitized slowly in cultured dorsal root ganglion neurons. Histamine-induced $[Ca^{2+}]_i$ was dependent on extracellular Ca^{2+} and inhibited by capsazepine and by SC0030, competitive antagonists of TRPV1. Quinacrine and nordihydroguaiaretic acid, a PLA_2 and an LO inhibitor, respectively, blocked the histamine-induced Ca^{2+} influx in sensory neurons, while indomethacin (a cyclooxygenase inhibitor) did not. We thus conclude that histamine activates TRPV1 after stimulating the PLA_2/LO pathway, leading to the excitation of sensory neurons. These results further provide an idea for potential use of TRPV1 antagonists as anti-itch drugs.
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Keywords: TRPV1; Histamine; Itch; Dorsal-root ganglion neuron; Phospholipase A_2; Lipoxygenase; Nociceptor

It is now known that histamine released from activated mast cells in the skin causes itch sensation [5,9,10]. Patients with atopic dermatitis whose histamine content in the skin is high complain of itch [17,18]. Histamine is known to excite a subset of unmyelinated afferent fibers in human skin [19, 20]. The intensity of itch after histamine application coincides well with activity of a subset of C-fibers [19]. Thus, several lines of psychophysical and electrophysiological evidence support that histamine is pruritogenic. However, mechanisms underlying histamine-mediated excitation of sensory neurons remain elusive.

TRPV1 is a non-specific cation channel that is preferentially present in small sensory neurons and activated by capsaicin, heat and acid [2,4,22]. Because inflammatory thermal hypoalgesia is produced in mice lacking TRPV1 [3, 7], TRPV1 is now considered as a molecular sensor that detects a painful condition such as inflammation. In line with this notion, proinflammatory signals appear to converge on TRPV1 [6,16]. One of inflammatory signals to TRPV1 is bradykinin, a major pain-causing substance. Recently, we have demonstrated that bradykinin activates TRPV1 via an intracellular second messenger pathway involving mobilization of arachidonic acid by phospholipase A_2 (PLA_2) and generation of an lipoxygenase (LO) product, 12-hydroperoxyeicosatetraenoic acid [21]. Lines of evidence suggest that TRPV1 would mediate the response of primary afferents to histamine. For example, capsaicin excites cutaneous nerve fibers that histamine excites [20]. Histamine sensitizes testicular afferent fibers to heat [12, 14]. Thus, it is likely that histamine activates TRPV1 in order to excite sensory neurons possibly via the PLA_2/LO pathway. Therefore, we sought to determine whether histamine could activate TRPV1 via stimulation of PLA_2 and LO. Since TRPV1 is permeable to Ca^{2+}, the activation of TRPV1 is assessed by measuring changes in $[Ca^{2+}]_i$.

Dorsal-root ganglion (DRG) neurons were cultured as described previously [11,21]. Briefly, DRGs were collected in the cold culture medium (4 °C) containing DMEM-F-12 mixture, 10% fetal bovine serum, 1 mM sodium pyruvate, 25–100 ng/ml NGF (Alomone Labs, Jerusalem, Israel), and 100 U/ml of penicillin-streptomycin (Sigma, St. Louis, MO). Ganglia were washed three times with DMEM-F-12 medium and incubated for 30 min in the DMEM-F-12 medium containing 1 mg/ml collagenase (type II, Worthington, Freehold, NJ). The ganglia were then washed three times with Mg^{2+}- and Ca^{2+}-free Hank's balanced salt

☆ This paper is in honor of Manfred Zimmermann's 70th birthday.

* Corresponding author. Tel.: +82-2-880-7854; fax: +82-2-872-0596.
E-mail address: utoh@plaza.snu.ac.kr (U. Oh).

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solution (HBSS; Life Technologies) and incubated with gentle shaking in the warm (37 °C) HBSS containing 2.5 mg/ml trypsin. Cells were then centrifuged at 1000 rpm for 10 min, resuspended in the culture medium and plated on glass-bottom culture dishes (Met Tek, Ashland, MA) that had been pretreated with poly-L-lysine. Cells were incubated at 37 °C in a 95% air-5% CO_2 gas mixture.

The intracellular calcium concentration ($[Ca^{2+}]_i$) was measured as described previously [21]. Briefly cultured DRG cells were loaded with a calcium-sensitive fluorescent dye, Fluo-3/AM (50 μM, Molecular Probes, Eugene, OR) for 30 min at 37 °C. After three-time-wash with control solution (see below), images were taken by a confocal microscope (Leica, Heidelburg, Germany) at a sample rate of 1 Hz. Histamine was applied to the cells during scanning. Fluo-3/AM was excited at the 488 nm. The excitation and emission lights were separated with a dichroic mirror at 510 nm. The emission light was truncated with a 515–545 nm band pass filter. For measurements of $[Ca^{2+}]_i$-dependent changes, a sequence of fluorescence images was sampled in a fixed focal plane. Regions of interest were defined in the first image, and the normalized fluorescence change, F/F_0 was measured throughout the image sequence where F_0 was the initial fluorescence intensity. Solution for Ca^{2+} imaging in cultured neurons contained 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Na-HEPES, and 10 mM glucose (pH 7.2). For Ca^{2+}-free solution, 5 mM EGTA was added instead of 1 mM $CaCl_2$. All values were expressed as mean ± SEM. Two means were compared with Student's unpaired *t*-test. *P* value less than 0.05 was considered significant.

When 10 μM of histamine was applied to cultured DRG neurons, a rapid increase of $[Ca^{2+}]_i$ was observed, which then declined slowly to the baseline level within 100 s (Fig. 1). About 51% (1138 out of 2210) cells responded to 10 μM of histamine challenge. The number of cells responding decreased when less amount of histamine was applied. When 1 and 5 μM of histamine were applied, 16.2 and 28.9% of DRG cells exhibited Ca^{2+} influx, respectively. Because proportion of the responsive cells saturated at 50 μM of histamine (data not shown), further experiments were carried out at 10 μM of histamine. Extracellular Ca^{2+} was necessary for the histamine-induced increase in $[Ca^{2+}]_i$ because the histamine effect was not observed in Ca^{2+}-free bath solution ($n = 15$) (Fig. 1B).

Because capsaicin and histamine excite a subgroup of nociceptors, capsaicin was applied to DRG neurons. Majority of cells (85.7%, 48 out of 56) that responded to histamine exhibited an increase in $[Ca^{2+}]_i$ after subsequent application of 0.5 μM capsaicin (Fig. 1C). Capsazepine, a competitive inhibitor of TRPV1 was applied to determine whether TRPV1 mediates the histamine-induced Ca^{2+} influx. Because the $[Ca^{2+}]_i$ increase by a histamine challenge was not sustained, the effects of various pharmacological inhibitors could not be properly tested with this protocol. Therefore, we applied 10 μM of histamine for 30 s twice in 3 min interval (Fig. 2A). The

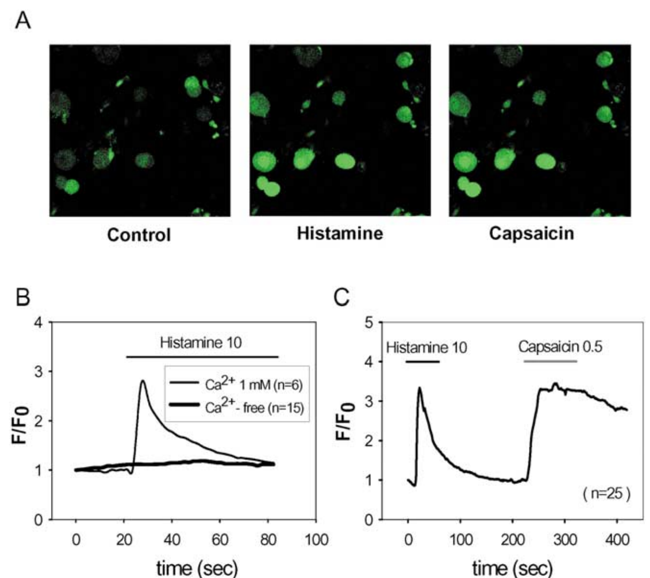

Fig. 1. The effect of histamine on cultured DRG neurons. (A) Confocal microscopic images of $[Ca^{2+}]_i$ response of DRG neurons before (control) and after histamine or capsaicin application. Cultured DRG neurons were pre-incubated with Fluo-3/AM before histamine application. (B) Effect of 10 μM of histamine on $[Ca^{2+}]_i$ in the bath containing 1 mM Ca^{2+} or Ca^{2+}-free solution (5 mM EGTA and 0 Ca^{2+}). $[Ca^{2+}]_i$ changes were expressed as the ratio F/F_0, where F_0 was the initial fluorescence intensity. Traces are the averaged Ca^{2+}-transient response of the number of cells indicated. The histamine concentration is expressed in μM. (C) Co-activation by histamine and capsaicin of sensory neurons. Histamine followed by 0.5 μM of capsaicin was applied to cultured DRG neurons. Traces are the averaged Ca^{2+}-transient response of the number of cells indicated.

magnitude of $[Ca^{2+}]_i$ induced by the second histamine challenge was compared with that of the first histamine challenge. In the control condition, the Ca^{2+}-influx to the second histamine challenge was smaller than the first response (66.6 ± 5.2%, $n = 12$). When 5 μM of capsazepine was pretreated, the histamine-induced Ca^{2+}-transient was significantly reduced ($P < 0.001$, 2.4 ± 1.5%, $n = 25$) (Figs. 2A,B). In contrast, pretreatment of vehicle to capsazepine did not affect the Ca^{2+}-transient evoked by the second histamine challenge ($n = 10$). Because capsazepine is known to affect activities of other channels as well [8,13], another TRPV1 competitive antagonist, SC0030 was applied [23]. Treatment of 5 μM of SC0030 prior to the second histamine application also completely blocked the histamine-induced Ca^{2+} influx ($P < 0.001$, −3.7 ± 1.2%, $n = 21$). These results clearly suggest that histamine induces Ca^{2+} influx by activating TRPV1.

Histamine receptors have three isotypes, H_1, H_2, and H_3 [1]. In order to determine which receptor mediates the histamine-induced increase of $[Ca^{2+}]_i$ in sensory neurons, we used histamine receptor antagonists relatively specific to each isotype, mepyramine (H_1 receptor antagonist), cimetidine (H_2 receptor antagonist), and thioperamide (H_3 receptor antagonist). As reported by Nicolson and his colleagues [15], only the H_1 receptor antagonist, mepyramine (1 μM), reduced the histamine-induced Ca^{2+}-influx significantly ($P < 0.001$,

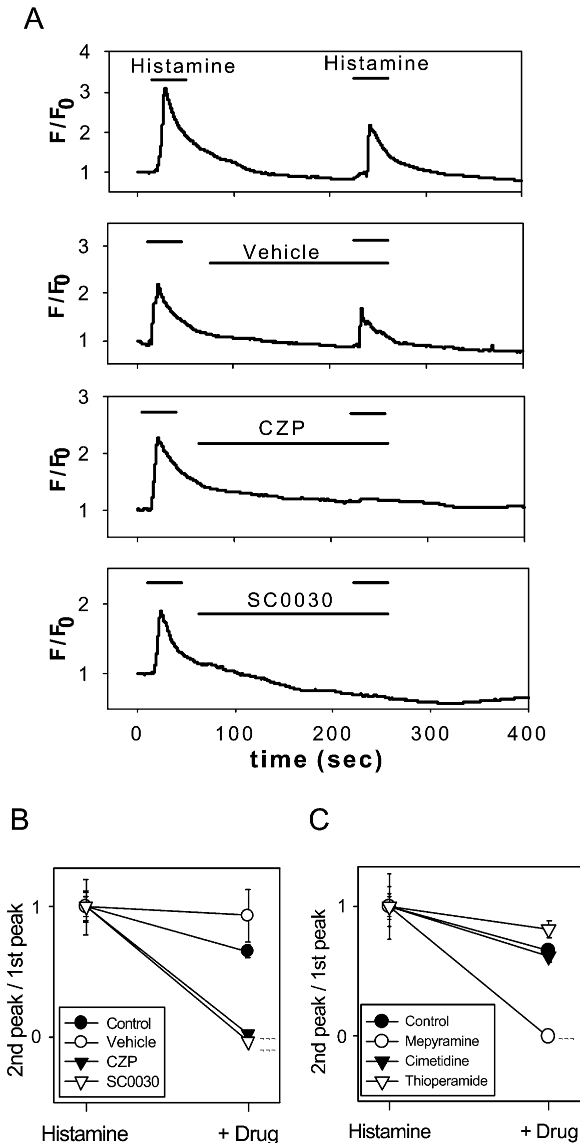

Fig. 2. The effects of TRPV1 antagonists, capsazepine (CZP) and SC0030, on the histamine-induced $[Ca^{2+}]_i$. Histamine (10 μM) was applied twice in 3 min interval. (A) Traces of averaged Ca^{2+}-trasients induced by histamine challenges. Capsazepine (CZP, 5 μM, $n = 25$), SC0030 (5 μM, $n = 21$), and vehicle ($n = 10$) were pretreated before the second histamine challenge. (B) Summary of the effects of TRPV1 antagonists. The magnitude of the second Ca^{2+}-transient peak was normalized with that of the first peak. ***, $P < 0.001$. (C) Summary of the effects of histamine receptor antagonists, mepyramine (H_1 antagonist, 1 μM, $n = 12$), cimetidine (H_2 antagonist, 50 μM, $n = 39$), and thioperamide (H_3 antagonist, 1 μM, $n = 35$), on the histamine-induced $[Ca^{2+}]_i$. ***, $P < 0.001$.

$-0.4 \pm 0.5\%$, $n = 33$). In contrast, 50 μM of cimetidine ($61.7 \pm 4.3\%$, $n = 39$) or 1 μM of thioperamide ($82.1 \pm 6.8\%$, $n = 35$) failed to reduce the Ca^{2+}-influx induced by the second histamine challenge (Fig. 2C). These results suggest that histamine increases $[Ca^{2+}]_i$ in cultured DRG neurons via activation of H_1 receptor.

To test the hypothesis that the PLA_2/LO pathway mediates the neuronal excitation by histamine, enzyme inhibitors were applied. As shown in Fig. 3, pretreatment of 10 μM of quinacrine, a PLA_2 inhibitor, significantly reduced the histamine-induced Ca^{2+} influx ($P < 0.001$, $0.4 \pm 0.6\%$, $n = 87$). Furthermore, 20 μM of nordihydroguaiaretic acid, a non-specific LO inhibitor, also significantly ($P < 0.001$, $1.6 \pm 0.5\%$, $n = 35$) reduced the histamine-induced Ca^{2+} influx. In contrast, 10 μM of indomethacin, a cyclooxygenase inhibitor failed to block the histamine-induced $[Ca^{2+}]_i$ ($71.5 \pm 4.4\%$, $n = 39$). These results indicate that histamine induces increase in $[Ca^{2+}]_i$ by stimulating PLA_2 and LO.

Histamine, a pruritogen, is known to excite cutaneous nociceptors, especially mechanically insensitive C-fibers

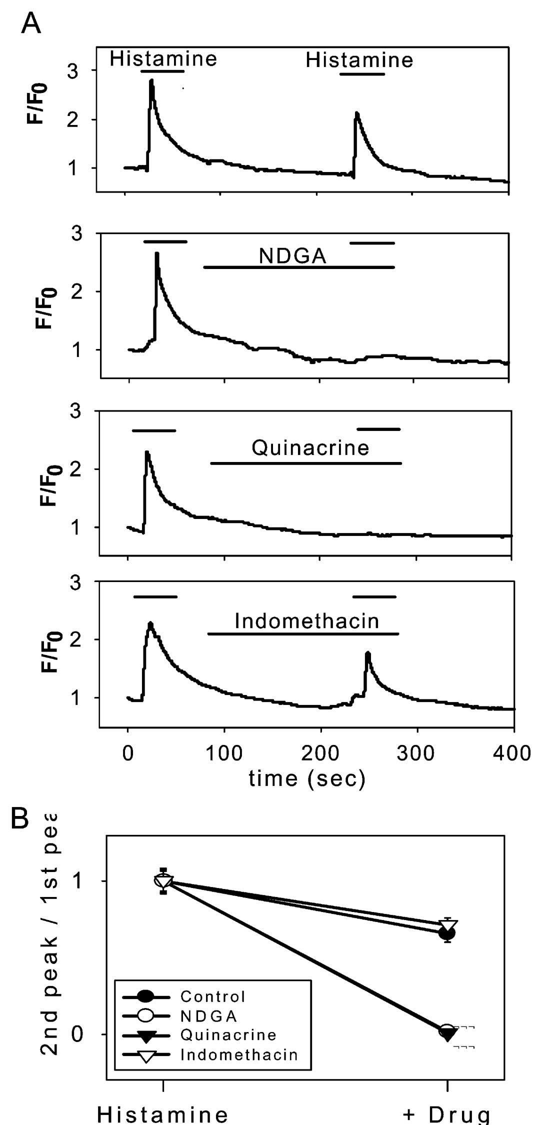

Fig. 3. The effects of PLA_2, LO, or cyclooxygenase inhibitor on the histamine-induced $[Ca^{2+}]_i$. (A) Traces of averaged Ca^{2+}-trasients induced by histamine challenges. Nordihydroguaiaretic acid (NDGA, 20 μM, $n = 35$), quinacrine (PLA_2 inhibitor, 10 μM, $n = 87$), and indomethacine (cyclooxygenase inhibitor, 10 μM, $n = 39$) were pretreated before the second histamine challenge. (B) Summary of the effects of NDGA, quinacrine, and indomethacine on the histamine-induced $[Ca^{2+}]_i$. ***, $P < 0.001$.

[20]. But until now, the action mechanism of histamine in sensory neurons is poorly understood. In the present study, we showed that histamine caused Ca^{2+} influx via opening of TRPV1 and by the actions of PLA_2 and LO. Inhibition of the histamine-induced Ca^{2+}-influx by TRPV1 antagonists or PLA_2 and LO inhibitors was specific because pretreatment with vehicle, indomethacin, cimetidine, or thioperamide failed to block the histamine response. Thus, this is the first study that suggests the possible histamine-induced intracellular signaling pathway in sensory neurons.

Capsaicin activates virtually all nociceptors in the human skin. Similarly, histamine excites a subgroup of nociceptors that respond to capsaicin [20]. In the present study, we also observed that the majority of histamine-sensitive neurons also respond to capsaicin. The high propensity of histamine and capsaicin-sensitive cells in part explains that action mechanism of histamine relates to TRPV1. However, in the present study, some of histamine-sensitive neurons still did not respond to capsaicin. Thus, histamine may also excite sensory neurons by alternative mechanisms that do not involve the PLA_2/LO/TRPV1 pathway, such as phospholipase C. Indeed, histamine is known to increase the production of inositol 1,4,5-triphosphate, an indication of the involvement of phospholipase C [15]. Furthermore, the protein kinase C pathway may involve the histamine-evoked response of primary afferents because inhibition of protein kinase C reduces neural response to histamine and sensitization of heat response evoked by histamine [14].

In summary, the present study demonstrates that histamine activates TRPV1, PLA_2 and LO in sensory neurons. However, identification of precise signaling mechanisms requires further studies. Nonetheless, the possible involvement of TRPV1 in mediating the histamine action on sensory neurons now suggests a clinical application of TRPV1 antagonists as possible anti-pruritogenic agents.

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