Stimulation of Histamine H₁ Receptor Up-Regulates Histamine H₁ Receptor Itself Through Activation of Receptor Gene Transcription

Asish K. Das¹, Sachiho Yoshimura¹, Ryoko Mishima³, Katsumi Fujimoto³, Hiroyuki Mizuguchi¹, Shrabanti Dev¹, Yousuke Wakayama¹, Yoshiaki Kitamura², Shuhei Horio¹, Noriaki Takeda³, and Hiroyuki Fukui¹,*

¹Department of Molecular Pharmacology and ²Department of Otolaryngology, Graduate School of Health Biosciences, The University of Tokushima, Tokushima 770-8505, Japan
³Department of Biochemistry, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima 734-8551, Japan

Received November 8, 2006; Accepted February 7, 2007

Abstract. Histamine is a major mediator in allergy acting mainly through the histamine H₁ receptor (H1R). Although H1R up-regulation has been suggested as an important step for induction of allergic symptoms, little is known about the regulation of H1R level. Here we report that the activation of H1R up-regulates H1R through augmentation of H1R mRNA expression in HeLa cells. Histamine stimulation significantly increased both H1R promoter activity and mRNA level without alteration in mRNA stability. H1R protein was also up-regulated by histamine. An H1R antagonist but not histamine H₂ receptor antagonist blocked histamine-induced up-regulation of both promoter activity and mRNA expression. A protein kinase C (PKC) activator, phorbol-12-myristate-13-acetate, increased H1R mRNA expression, whereas an activator of PKA or PKG (8-Br-cAMP or 8-Br-cGMP, respectively) did not. Furthermore, histamine-induced up-regulation of both promoter activity and mRNA level were completely suppressed by the PKC inhibitor Ro-31-8220. H1R antagonists have long been thought to block H1R and inhibit immediate allergy symptoms. In addition to this short-term effect, our data propose their long-term inhibitory effect against allergic diseases by suppressing PKC-mediated H1R gene transcription. This finding provides new insights into the therapeutic target of H1R antagonist in allergic diseases.

Keywords: histamine, histamine H₁ receptor, receptor gene transcription, protein kinase C, HeLa cell

Introduction

Histamine plays important pathophysiological roles in central and peripheral tissues (1). The pathophysiological function of histamine is mediated through four distinct G protein-coupled receptors that are classified as H₁, H₂, H₃, and H₄ (1, 2). Histamine H₁ receptor (H1R) is a seven transmembrane-spanning receptor coupled with the G_{q/11} family of G proteins (3). Activation of this receptor leads to stimulation of phospholipase C that catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to form inositol 1,4,5-trisphosphate and diacylglycerol. Inositol 1,4,5-trisphosphate released into the cytoplasm causes the mobilization of calcium from intracellular stores, whereas diacylglycerol activates protein kinase C (PKC) (1).

Several studies have documented that the expression of H1R mRNA changes in pathological situations. Increased expression of H1R mRNA was observed in the nasal scrapings of patients with allergic rhinitis (4) and in cultured epithelial, mucus, and inflammatory cells in complex tissues of the nasal mucosa of perennial allergic rhinitis (5). Diesel exhaust particulates, the common air pollutant from diesel engine-powered car exhaust, responsible for chronic airway diseases also up-regulates H1R mRNA (6).
Our previous works (7, 8) have demonstrated that repeated intranasal application of toluene 2,4-diisocyanate (TDI) induced release of histamine from mast cells via neurogenic inflammation and leads to the development of nasal hypersensitivity behavior in guinea pigs. We also showed that both H1R mRNA and H1R protein level were up-regulated in TDI sensitized allergic rats and H1R mRNA was significantly suppressed by the treatment of d-chlorpheniramine, an H1R antagonist (9). These studies suggest that H1R gene expression is closely related to the pathological condition of allergy and that the activation of H1R is involved in H1R up-regulation.

In this study, we sought to determine whether H1R activation is involved in H1R up-regulation in HeLa cells expressing endogenous H1R. Our results indicate that the stimulation of H1R by histamine induces up-regulation of H1R mRNA expression via the PKC pathway and thereby increases H1R protein level.

Materials and Methods

Materials

[Pyridinyl-5-3H]mepyramine (specific activity: 20 Ci /mmol) was obtained from Perkin Elmer (Boston, MA, USA). PolyFect transfection reagent was from Qiagen K.K. (Tokyo). The dual-luciferase reporter assay system and pRL-MPK vector were from Promega (Madison, WI, USA). Pre-developed TaqMan Assay Reagent of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was from Applied Biosystems (Foster City, CA, USA). MEM-alpha medium was from Gibco (Grand Island, NY, USA). TRIzol reagent was purchased from GIBCO-BRL (Carlsbad, CA, USA). Ro-31-8220 was purchased from CALBIOCHEM (San Diego, CA, USA). The BCA protein assay kit was from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Cells

HeLa cells were cultured at 37°C in a humidified 5%-95% CO2-air atmosphere in MEM-alpha medium containing 8% fetal calf serum (Sigma) and supplemented with 100 IU/ml penicillin (Sigma) and 50 µg/ml streptomycin (Sigma).

[3H]Mepyramine binding assay

HeLa cells incubated with various drugs for indicated times were subjected to the [3H]mepyramine binding assay as described previously (10, 11). In brief, cells were harvested with 1.5 ml of ice-cold 50 mM Na2/K-phosphate buffer (37.8 mM Na2HPO4, 12.2 mM KH2PO4, pH 7.4). The cells were homogenized with an ultrasonic disrupter, and the homogenate was centrifuged at 45,000 x g for 30 min at 4°C. The pellet was resuspended in ice-cold 50 mM Na2/K-phosphate buffer and served as membrane sample for radioligand binding assay. Membranes were incubated with 4 nM of [3H]mepyramine in the absence or presence of 10 μM triprolidine for 60 min at 25°C in a final volume of 500 µl. The reaction was terminated by rapid vacuum filtration through Whatman GF/B filters presoaked with 1% polyethyleneimine. Filters were soaked in 10 ml of Aquasol-2, kept overnight in a dark place, and the radioactivity trapped on the filters was counted in a liquid scintillation counter. Specific binding was defined as the radioactivity bound after subtraction of nonspecific binding as defined by the binding in the presence of 10 μM triprolidine. Protein concentration was determined by the BCA protein assay reagent using BSA as a standard.

Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR)

HeLa cells cultured to 70% confluency in 35-mm dishes were serum starved for 24 h and were treated with different reagents for the indicated time. After the treatment, the cells were harvested with 700 µl of TRIzol reagent, mixed with 140 µl of chloroform, and centrifuged at 15,000 rpm for 15 min at 4°C. The aqueous phase was collected and the RNA was precipitated by addition of isopropanol. After centrifugation at 15,000 rpm for 15 min at 4°C, the resulting RNA pellet was washed with ice-cold 70% ethanol. Total RNA was resolved in 10 µl of diethylpyrocarbonate-treated water and 5 µg of each RNA sample was used for reverse transcription reaction in a 25 µl of reaction buffer containing 375 mM KCl, 250 mM Tris-HCl (pH 8.3), 15 mM MgCl2, 0.8 mM of each of the deoxyribonucleoside triphosphates (dNTPs), 6.7 μM oligo (dT) primers, 0.1 units of RNase inhibitor (Wako, Tokyo), and 200 units of SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The reaction mixtures were incubated for 60 min at 37°C. Then 1.25 µl of 2 N NaOH was added to the reaction mixture and further incubated for 30 min at 65°C. After incubation, 8 µl of 1 M Tris-HCl (pH 8.0) was added and the samples were heated for 10 min at 95°C and then chilled at 4°C for 5 min. The transcripts (3 µl) were amplified by using the GeneAmp 5700 Sequence Detection System (Applied Biosystems) in 28 µl of reaction mixture containing 3 mM MgCl2, 660 nM of both forward and reverse primer, 330 nM of probe, 14 µl of Platinum Quantitative PCR Super-Mix UDG (Invitrogen). TaqMan primers and probe were designed using Primer Express software (Applied Biosystems).
The sequences of the primers and TaqMan probe were as follows: forward primer for H1R, 5'-CAGAGGATC AGATGTTAGGTGATAGC-3'; reverse primer for H1R, 5'-AGCGGAGCCTCTTCCAAGTAA-3'; TaqMan probe, FAM-CTTCTCTCGAAGGACTCAGATACC ACC-TAMRA. To standardize the starting material, human GAPDH gene was used. Data were expressed as the ratio of H1R mRNA and GAPDH mRNA. Amplicon size and reaction specificity were confirmed by agarose gel electrophoresis using 2% agarose gel containing 0.5 µg/ml ethidium bromide. The identity of the PCR products was verified by sequencing using a DNA sequencer (Aloka, Tokyo).

**H1R promoter assay**

Human H1R reporter plasmid (pH1R) was constructed as follows: A 2.1-kbp 5'-upstream fragment [−2029 to +64, +1 indicates the putative transcription initiation site (12) of human H1R gene] was amplified by PCR using a forward primer (5'-GCTAGCGGAAT GTGGGAAGCCTCTTCCAAGTAA-3') and a reverse primer (5'-AGATCTGAAAGTCTTCCATGATGGGCTTC-3') and then ligated to pGEM-T Easy vector (Promega). After confirming the sequence, the fragment was then subcloned into the NheI-BglII site of the promoter-less luciferase reporter plasmid pGL3-Basic vector (Promega).

HeLa cells cultured in 12-well culture plates were co-transfected with pH1R and the internal control plasmid pRL-MPK containing cDNA encoding mouse pyruvate kinase and Rluc (Promega) by the ratio of 100:1 using PolyFect transfection reagent (Qiagen) according to the manufacturer’s instructions. After 5 h, the medium was replaced with 1 ml of serum-free medium and starved for 24 h. The cells were stimulated with appropriate reagents for the indicated time in the same medium. Where appropriate, antagonists were added 30 min prior to histamine stimulation. After stimulation, cells were washed twice with 500 µl of ice-cold phosphate-buffered saline and lysed with 100 µl of passive lysis buffer (Promega). The lysate was frozen for at least 3 h at −85°C and then thawed at room temperature. The luciferase activity was determined by the dual-luciferase reporter assay system (Promega) as per manufacturer’s protocol. Luminescence was measured by photoluminescence reader BLR 302 (Aloka). The measurement was integrated over 20 s with no delay.

**Statistical analysis**

All data are expressed as the mean ± S.E.M. of at least three independent experiments carried out in triplicate. Statistical analysis was carried out by the unpaired Student’s t-test. P-values <0.05 were considered statistically significant.

**Results**

**Effect of histamine on H1R, H1R mRNA, and promoter activity**

Treatment of HeLa cell with 10 µM of histamine caused significant up-regulation of H1R expression level and it was about 1.4-fold higher than that in the control cells after 24 h of stimulation (Fig. 1A). To test whether histamine-induced up-regulation of H1R was derived by an increase in the H1R mRNA level, we carried out real-time RT-PCR to determine the H1R mRNA level in the histamine-stimulated HeLa cells. H1R mRNA was measured at different time intervals after stimulation with 10 µM histamine. Histamine induced a transient and time-dependent increase of H1R mRNA (Fig. 1B). Following 9-h treatment, the mRNA level reached maximum (1.5-fold over the control level) and afterwards declined gradually, returning near to the basal level after 24-h treatment. The stability assay showed that histamine had no effect on H1R mRNA stability (Fig. 2). These data suggest that the histamine-induced increase of the H1R mRNA may be due to the activation of H1R promoter activity. To confirm this, HeLa cells were co-transfected with pH1R and pRL-MPK vectors. Following stimulation with 10 µM of histamine, the H1R promoter activity was measured at different time intervals by the dual-luciferase reporter assay. As shown in Fig. 1C, histamine caused a marked increase in H1R promoter activity, and it was 2.5-fold over the control level after 12 h of histamine treatment.

**Inhibition of histamine-induced up-regulation of H1R mRNA, and promoter activity by histamine-receptor antagonists**

To test whether histamine-induced increases in H1R mRNA and H1R promoter activity are mediated through H1R, we investigated the effect of an H1R antagonist on the above mentioned parameters. Real-time RT-PCR studies suggested that 1-h pretreatment with 10 µM of d-chlorpheniramine completely blocked the histamine-induced up-regulation of H1R mRNA level (Fig. 3A). In the promoter assay, pretreatment with 10 µM of d-chlorpheniramine also completely reversed the histamine-induced up-regulation of H1R promoter activity (Fig. 3B). However, pretreatment with the histamine H2-receptor antagonist, ranitidine (10 µM) had no effect on histamine-induced up-regulation of H1R mRNA (Fig. 4). These results indicated that histamine-induced up-regulations of H1R mRNA and H1R promoter activity are mediated through H1R.
PKC pathway is involved in histamine-induced up-regulation of H1R gene expression

H1R is a Gq-coupled receptor and causes the activation of PKC. However, it has been unclear up to now whether other protein kinases are involved in H1R gene expression. This prompted us to assess the effects of phorbol-12-myristate-13-acetate (PMA), a PKC activator; 8-Br-cAMP, a protein kinase A (PKA) activator; and 8-Br-cGMP, a selective protein kinase G (PKG) activator, on the histamine-induced H1R expression, H1R mRNA level, and H1R promoter activity by means of the \([3H]\)mepyramine binding assay, real-time RT-PCR, and dual-luciferase reporter assay, respectively. 8-Br-cAMP and 8-Br-cGMP induced no up-regulation but rather decreased the promoter activity and gene expression level to about 70% of the control. However, HeLa cells treated for 24 h with 1 µM PMA showed 150% H1R protein expression compared with that of the control (Fig. 5A). Real-time RT-PCR data showed that PMA had a transient and time-dependent stimulatory effect on H1R gene expression (Fig. 5B). To check the involvement of PKC on H1R promoter activity, we carried out the dual-luciferase reporter assay. HeLa cells were treated with 1 µM PMA for 4 h before determination of the luciferase activity. As shown in Fig. 5C, PMA caused a 4.3-fold increase in the
promoter activity compared with that of the control. We analyzed the effect of PKC inhibitor on histamine-induced up-regulation of H1R gene expression. HeLa cells were treated with 1 μM of d-chlorpheniramine for 1 h before the histamine (10 μM) stimulation. As shown in Fig. 6A, Ro-31-8220 completely inhibited histamine-induced up-regulation of H1R gene expression. Similar results were obtained in the H1R promoter assay (Fig. 6B). These data indicate that the PKC pathway, not PKA or PKG, is involved in determining the H1R expression, the H1R mRNA level, and the H1R promoter activity.

Discussion

In this report, we have demonstrated that stimulation of H1R regulates the level of H1R protein expression through activation of H1R mRNA synthesis. To search for the regulatory mechanisms of H1R, we used HeLa cells expressing endogenous H1R. The H1R level could be regulated by various processes including modulation of receptor gene transcription, mRNA stability, and receptor degradation.

In our previous study, we showed that neurogenic inflammation caused histamine release from mast cells in nasal mucosa and led to the development of nasal hypersensitivity in TDI-sensitized allergic rats (13). We also reported that the H1R mRNA level in TDI rats was significantly higher than that of control animals (13). These findings were in line with studies in which the expression of H1R mRNA was increased in epithelial and endothelial cells of nasal mucosa (14), in neural cells (15), and in the nasal scrapings from patients with nasal allergy (4). It has also been reported that H1R binding activity in the nasal mucosa is increased with the development of nasal allergy (16). In addition, we showed the up-regulation of H1R in the nasal mucosa of TDI rats (9, 13). Furthermore, pretreatment with d-
chlorpheniramine, an H1R antagonist, significantly suppressed the TDI-induced increase in H1R mRNA level (9). These studies suggested that up-regulation of the H1R mRNA level is mediated through H1R.

Stimulation of HeLa cells with histamine induced up-regulation of H1R and it was 40% over the control level after 24 h (Fig. 1A). Histamine induced significant and transient increase in H1R mRNA and the maximum level was achieved after 9-h stimulation (Fig. 1B). The kinetics of the histamine-induced increase in H1R mRNA level and H1R expression level indicate that the increased H1R mRNA leads to an increase of receptor...
translation and receptor expression. H1R promoter activity was also increased after histamine stimulation (Fig. 1C), but the stability of H1R mRNA was not affected by the stimulation with histamine (Fig. 2), indicating that the up-regulation of H1R was a consequence of an increase in H1R mRNA synthesis. Compared with the degree of the histamine-induced H1R transcriptional activity, the level of the histamine-induced $[^3H]$mepyramine binding activity seems to be somewhat low. It is likely that this low $[^3H]$mepyramine binding activity is due to the increased degradation of H1R by the homologous H1R stimulation. Previously, we have shown that stimulation with histamine abolished 60% of the H1R expression in CHO cells expressing recombinant H1R (CHO-H1) compared with the non-stimulated cells (17). So one could easily speculate that stimulation of HeLa cells with histamine causes a similar decrease in $[^3H]$mepyramine binding activity. However, elevation of $[^3H]$mepyramine binding activity was observed in HeLa cells. This indicates the strong effect of histamine on activation of the H1R gene transcription in HeLa cells. Similar cell type-dependent opposite regulation of gene expression was reported for the melanocortin-1 receptor (18). As shown in Fig. 3, A and B, H1R mRNA and promoter activity was observed in HeLa cells in the absence of histamine. This is likely due to the constitutive activity of H1R as it has been reported that H1R is a constitutively active (19). Pretreatment with $d$-chlorpheniramine down-regulated the basal level of both H1R promoter activity and H1R mRNA. It may be due to the inverse agonistic activity of $d$-chlorpheniramine. It also inhibited even the agonist-induced up-regulation of both promoter activity and gene expression and maintained at below the basal level, whereas pretreatment with ranitidine failed to show any effect on histamine-induced up-regulation of H1R mRNA. Our data clearly show that histamine-induced up-regulation of H1R is through increased receptor gene transcription due to enhanced promoter activity. To our knowledge, this is the first report indicating H1R-mediated H1R up-regulation.

We subsequently examined the role of different protein kinases on H1R-mediated H1R gene expression. PKA and PKG activation failed to produce any significant effect on H1R promoter activity and H1R gene expression (data not shown), whereas the PKC activator PMA caused a significant increase in both H1R promoter activity and H1R gene expression (Fig. 5: A and B), indicating the involvement of PKC in the up-regulation of H1R. Ro-31-8220 was able to inhibit completely both H1R promoter activity and H1R mRNA synthesis (Fig. 6: A and B), confirming the role of PKC in the downstream responses of the H1R signaling.
H1R-Mediated H1R Up-Regulation

pathway between PKC and H1R promoter activity, but speculate that MAP kinase and/or the NF-xB pathway may be the possible target of PKC for elevating H1R promoter activity and gene expression.

The increasing prevalence of allergic rhinitis, its impact on individual quality of life and social costs, as well as its role as a risk factor for asthma, underline the need for improved treatment options for this disorder. H1Rs are involved in the pathologic processes of allergy and H1R antagonists are one of the most often used classes of drugs to relieve the symptoms of allergic diseases such as allergic rhinitis, atopic dermatitis, psoriasis, allergic conjunctivitis, and so on (1, 38 – 43). It has been suggested that a signal from H1R contributes to the antigen–receptor-mediated signaling pathways that induce proliferative responses and lead to the production of cytokines and antibodies by T cells and B cells, respectively (44). It would appear, therefore, that the H1R also has a wider role in the inflammatory process. So for developing better therapy, the regulation mechanism of H1R should be well understood. The present study shows that H1R does not simply mediate the action of histamine but also increases the number of H1R and so that intensifies H1R-mediated pathogenesis. As it is a continuous process under the presence of histamine, this finding justifies the treatment of allergy with H1R antagonists that not only block the H1R but also inhibit the up-regulation of the H1R mRNA, subsequently H1R protein expression, and ultimately decrease the nasal hypersensitivity to histamine.

In conclusion, we have shown that stimulation of H1R induced up-regulation of H1R through PKC-dependent activation of H1R gene transcription. This finding may help in the search for better drugs for allergy treatment. A detailed investigation must be devoted to defining the precise molecular mechanism behind this homologous up-regulation.

Acknowledgments

This work was financially supported in part by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (18390167 to H.F.) and by a fund from the Osaka Medical Research Foundation for Incurable Diseases.

References

18. Foroidevaux S, Eberie AN. Homologous regulation of melano-


