0022-3565/03/3053-1212–1221$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 305:1212–1221, 2003

Vol. 305, No. 3
46581/1064319
Printed in U.S.A.

Histamine H_4 Receptor Mediates Chemotaxis and Calcium Mobilization of Mast Cells

CLAUDIA L. HOFSTRA,[1] PRAGNYA J. DESAI, ROBIN L. THURMOND, and WAI-PING FUNG-LEUNG

Johnson and Johnson Pharmaceutical Research and Development LLC, San Diego, California

Received November 5, 2002; accepted February 25, 2003

ABSTRACT

The diverse physiological functions of histamine are mediated through distinct histamine receptors. Mast cells are major producers of histamine, yet effects of histamine on mast cells are currently unclear. The present study shows that histamine induces chemotaxis of mouse mast cells, without affecting mast cell degranulation. Mast cell chemotaxis toward histamine could be blocked by the dual H_3/H_4 receptor antagonist thioperamide, but not by H_1 or H_2 receptor antagonists. This chemotactic response is mediated by the H_4 receptor, because chemotaxis toward histamine was absent in mast cells derived from H_4 receptor-deficient mice but was detected in H_3 receptor-deficient mast cells. In addition, Northern blot analysis showed the expression of H_4 but not H_3 receptors on mast cells. Activation of H_4 receptors by histamine resulted in calcium mobilization from intracellular calcium stores. Both Gαi/o proteins and phospholipase C (PLC) are involved in histamine-induced calcium mobilization and chemotaxis in mast cells, because these responses were completely inhibited by pertussis toxin and PLC inhibitor 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U73122). In summary, histamine was shown to mediate signaling and chemotaxis of mast cells via the H_4 receptor. This mechanism might be responsible for mast cell accumulation in allergic tissues.

Histamine is a biogenic amine playing an important role in the regulation of different physiological processes in the body. Histamine is synthesized from L-histidine by histidine decarboxylation in specific cell types, such as mast cells, basophils, enterochromaffin-like cells, and neurons. The diverse biological effects of histamine are mediated through different histamine receptors, which are all G protein-coupled receptors. Almost a century of extensive pharmacological research using specific histamine receptor agonists and antagonists has identified three histamine receptors (H_1, H_2, and H_3 receptor). Each receptor has its own expression pattern and mediates distinct effects: H_1 receptors trigger smooth muscle contractions and are generally thought to play an important role in allergy, H_2 receptors regulate gastric acid secretion in the stomach, and H_3 receptors control the release of histamine and neurotransmitters by neurons (Hill et al., 1997). However, not all effects of histamine can be attributed to these three histamine receptors. Therefore, it has been suggested that another histamine receptor might exist (Raible et al., 1994). The molecular identity of this fourth human histamine receptor (H_4 receptor) was revealed recently (Oda et al., 2000; Liu et al., 2001a; Morse et al., 2001; Nguyen et al., 2001; Zhu et al., 2001), and subsequently, the H_4 receptor in mouse, rat, and guinea pig were cloned (Liu et al., 2001b).

The amino acid sequence of the H_4 receptor has low homology with other histamine receptors. Its closest member in the histamine receptor family is the H_3 receptor that shares only a 35% amino acid homology with the H_4 receptor, although the homology in the transmembrane region is 58%. However, the H_4 receptor expression pattern is distinct from the H_3 receptor. Although the expression of the H_3 receptor is mainly restricted to cells in the central nervous system (Lovenberg et al., 2000), the H_4 receptor seems to be limited to cells of the hemopoietic lineage (Oda et al., 2000; Liu et al., 2001a; Morse et al., 2001; Zhu et al., 2001). The expression of H_4 receptors on hemopoietic cells is not unique among the histamine receptor family, because T cells and dendritic cells also express H_1 and H_2 receptors (Jutel et al., 2001; Szeberenyi et al., 2001).

Pharmacological properties of the H_4 receptor have been revealed using H_4 receptor-transfected cells (Oda et al., 2000; Liu et al., 2001a; Morse et al., 2001; Nguyen et al., 2001; Zhu

[1] Current address: NV Organon, Molenstraat 110, P.O. Box 20, 5340 BH Oss, The Netherlands. E-mail: claudia.hofstra@organon.com
Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
DOI: 10.1124/jpet.102.046581.

ABBREVIATIONS: PTX, pertussis toxin; $H_3R-/-$, H_3 receptor gene knockout; $H_4R-/-$, H_4 receptor gene knockout; RT, reverse transcription; PCR, polymerase chain reaction; bp, base pair(s); Th, T helper; Tc, T-cytotoxic cells; FCS, fetal calf serum; IL, interleukin; LTB_4, leukotriene B_4; BSA, bovine serum albumin; PBS, phosphate-buffered saline; DNP-HSA, dinitrophenyl human serum albumin; IP_3, inositol 1,4,5-triphosphate; U-73122, 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione; U-73343, 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-2,5-pyrrolidine-dione.

et al., 2001). It was shown that specific H_1 and H_2 receptor antagonists and agonists do not bind to the H_4 receptor. However, more typical H_3 receptor ligands (such as thioperamide, clobenpropit, imetit, and *R*-α-methylhistamine) could bind the H_4 receptor with affinities different from that of the H_3 receptor.

Similar to other G protein-coupled receptors, histamine receptors activate specific G proteins that lead to the activation of signal transduction pathways (for review, see (Leurs et al., 1995). It has been shown that H_1 receptors mediate this action through Gαq proteins, resulting in calcium mobilization, H_2 receptors signal through Gαs proteins, and cAMP increase, whereas H_3 receptors signal through Gαi/o proteins and inhibition of cAMP (Lovenberg et al., 1999). In the literature, two signaling pathways are thought to be used by the H_4 receptor. First, using cells transfected with the H_4 receptor and a cAMP-responding reporter construct, studies have shown that histamine could inhibit forskolin-stimulated cAMP increases (Oda et al., 2000; Liu et al., 2001a; Zhu et al., 2001). However, this cAMP inhibitory effect is low in comparison with that mediated by the H_3 receptor. Second, one study showed that histamine could not alter cAMP levels in H_4 receptor-transfected cells, but instead increased calcium mobilization if the cells were cotransfected with Gαq/i1/2, Gαq/i3, or Gα16 proteins (Morse et al., 2001). The same study showed that histamine induced mitogen-activated protein kinase phosphorylation, which was inhibited by pertussis toxin (PTX). However, signaling pathways mediated by endogenous H_4 receptors have not been studied.

Mast cells are important effector cells in allergic diseases. Mast cells bind IgE with IgE receptor, and subsequent contact with antigens will trigger IgE receptor cross-linking and the release of preformed mediators, such as serotonin and histamine, and de novo-produced mediators, such as prostaglandins and leukotrienes. Although mast cells are best known for their histamine-releasing capacity, little is known about the effect of histamine on mast cells themselves.

In the present study, the expression pattern of mouse H_4 receptor on various purified hematopoietic cells and in various tissues was investigated. We showed that the mouse H_4 receptor was expressed specifically on eosinophils and mast cells. Bone marrow-derived mast cells were used to study the functional aspects and signaling pathways of the endogenous mouse H_4 receptor.

Materials and Methods

Human HMC-1 and WEHI-3 cells were purchased from American Type Culture Collection (Manassas, VA). Human $CD34^+$ cord blood cells were from AllCells, LLC (Berkeley, CA). Basophil enrichment kit and serum-free medium were from Stem Cells Technologies (Vancouver, BC, Canada). RNeasy kit was from QIAGEN (Valencia, CA). RT reaction kits and ExpressHyb solution were from BD Biosciences Clontech (Palo Alto, CA). A cAMP detection, nylon blot (Hybond), and Rediprime II kit were from Amersham Biosciences Inc. (Piscataway, NJ).

Thapsigargin, U73122 and U73343 were purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA). Transwells were purchased from Costar (Cambridge, MA). LTB_4 and prostaglandin detection kits were from Cayman Chemicals (Ann Arbor, MI). Fluo-3 was from TEF Labs (Austin, TX), and Pluronic acid was from Molecular Probes (Eugene, OR). Pertussis toxin and anti-DNP IgE was from ICN Pharmaceuticals (Costa Mesa, CA). All other antibodies were from BD PharMingen (San Diego, CA). Polylysine-coated black wall 96-well tissue culture plates were purchased from BD Biosciences (San Jose, CA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Methods

Generation of H_4 Receptor Gene Knockout Mice (H_4 R−/−). H_4R−/− mice were generated by Lexicon Genetics (Woodlands, TX). A 9-kb mouse genomic fragment containing the mouse H_4 receptor gene was obtained from the embryonic stem cell line 2G9 and was used as a template to prepare the knockout construct. A 0.5-kb region covering most of exon 1 and part of intron 1 of the H_4 receptor gene was deleted from this genomic fragment and replaced with a neomycin-resistant gene cassette. Homologous recombination in embryonic stem cells was confirmed by Southern analysis using a 3′ external probe amplified from the mouse H_4 receptor gene with oligonucleotides 5′-GAG ATG TAG ATG TGG TCG TTT G and 5′-CAT GTG CAG GCA CAC ACA TAC. The Southern blot of ES cell DNA digested with *Nco*I produced a 4.9-kb wild-type band and a 3.4-kb targeted band (Fig. 1). Chimeric mice were generated from embryos injected with embryonic stem cells. Germline mice were obtained by breeding chimeric male mice with C57BL/6 females. Germline mice heterozygous for the disrupted H_4 receptor gene were identified by PCR. Wild-type and H_4 R−/− mice were obtained from cross-breeding of heterozygous mice.

Detection of Mouse H_4 Receptor Expression. RNA from tissues and purified cells was prepared using a RNeasy kit according to the manufacturer's instructions. H_4 receptor RNA was detected by RT-PCR using specific mouse H_4 receptor primers (5′-ATG TCG GAG TCT AAC AGT ACT GG and 5′-AGA AGA TAC TGA CTG GTT CTG TGA). RT products of multiple tissues and cell types were amplified by PCR under conditions of 94°C 45 s, 55°C 45 s, and 72°C 2 min for 35 cycles. The PCR products were run on a 1% agarose gel with ethidium bromide (10 μg/ml), and DNA was visualized with UV light. The amplified mouse H_4 receptor cDNA is 1185 bp in size.

Mouse Th1 cells, Th2 cells, Tc1 cells, Tc2 cells, B cells, and macrophages were activated, and total RNA was prepared as described previously (Shier et al., 2000). Eosinophils were in vitro differentiated from C57BL/6J mouse bone marrows. Bone marrow was aseptically isolated from the femurs. The cells (2×10^5/ml) were cultured at 37°C with 5% CO_2 in RPMI 1640 culture medium consisting of 10% FCS, 0.1 mM nonessential amino acids, 50 μg/ml penicillin/streptomycin, 0.2 ng/ml IL-3, 0.4 ng/ml IL-5, and 0.2 ng/ml granulocyte-macrophage colony-stimulating factor. After 6 days, the me-

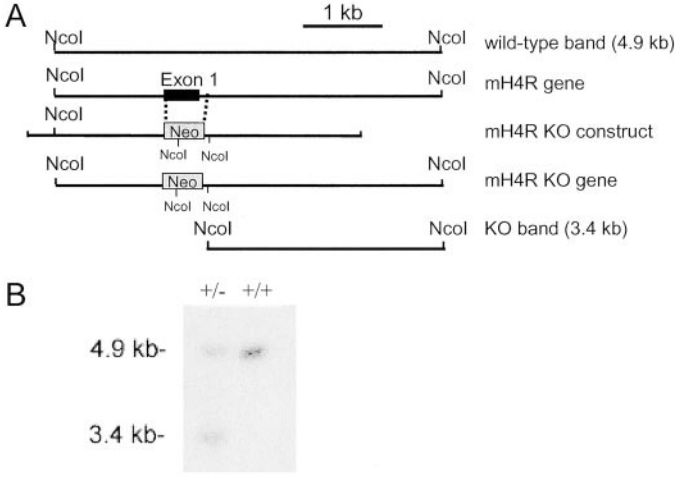

Fig. 1. Generation of H_4R−/− mice. A, mouse H_4 receptor gene was disrupted by replacing exon 1 with a neomycin-resistant gene cassette. B, disrupted H_4 receptor gene in H_4R+/− and H_4R+/+ mice was confirmed by Southern blot hybridization. A 4.9-kb DNA band from the wild-type gene and a 3.4-kb DNA band from the disrupted gene were shown.

dium was refreshed and cells were cultured for seven more days. Cells were stained with hematoxylin-eosin dyes and >95% of the cells displayed eosinophil phenotype. Mouse kidney, liver, thymus, spleen, lung, and brain were isolated from C57BL/6J mice. RNA was isolated from the tissues as described above.

Total RNA samples (5 μg) were run on a RNA gel and then transferred overnight to a nylon blot. The blot was prehybridized with ExpressHyb solution for 30 min at 68°C. The mouse H_4 receptor cDNA clone (Liu et al., 2001a) and the purified RT-PCR product of the mouse H_3 receptor (Liu et al., 2001b) were labeled using the Rediprime II kit. The blot was hybridized for 2 h at 68°C, followed by one wash (2× standard saline citrate and 0.05% SDS) of 40 min at room temperature, and a second wash (0.1× standard saline citrate and 0.1% SDS) of 40 min at 50°C. The blots were exposed to X-ray film at −70°C with two intensifying screens for 16 h.

Detection of Human H_4 Receptor Expression. Human basophils (98% purity) were isolated from human peripheral blood mononuclear cells using a basophil enrichment kit. Human mast cells were differentiated from human $CD34^+$ cells purified from cord blood, using serum-free medium supplemented with human stem cell factor (SCF) (100 ng/ml) and IL-6 (50 ng/ml) (Dahl et al., 2002). Cells were grown for 12 to 14 weeks, and media supplemented with cytokines was changed once a week. Cells were monitored weekly for their mast cell properties, using Geimsa, toluidine blue, tryptase, and CD117 staining. The cells showed metachromatic granule staining properties, as early as 2 to 3 weeks and by week 12, the purity of these cells for mast cell properties was 95%. Human mast cell line HMC-1 was cultured in Iscove's medium containing 10% FCS, 2 mM glutamine, and 1.2 mM monothioglycerol. Total RNA was extracted from human basophils, mast cells, and HMC-1 cells using an RNeasy kit, and 250 ng of RNA was used for the RT reaction according to manufacturer's instructions. PCR using human H_4 receptor-specific primers (5′-ACT AGA ATT CGC CAC CAT GCC AGA TAC TAA TAG CAC and 5′-ATG CAG GAT CCA GCA TTT GAG ACT GAC AGG TAT) was carried out as described previously (Liu et al., 2001a).

Bone Marrow Mast Cell Culture. Mast cells were differentiated from bone marrows collected from H_4 receptor gene knockout ($H_4R-/-$), H_3 receptor gene knockout ($H_3R-/-$) mice (Toyota et al., 2002), wild-type mice, and BALB/c and C57BL/6J mice. Bone marrow was aseptically isolated from the femurs. The cells (5×10^5/ml) were cultured at 37°C with 5% CO_2 in RPMI 1640 culture medium consisting 10% FCS, 0.1 mM nonessential amino acids, 50 μg/ml penicillin/streptomycin, and 20% WEHI-3 conditioned medium. WEHI-3 cells were cultured in Iscove's Dulbecco's medium with 10% FCS, 4 mM L-glutamine, 1.5 g/l sodium carbonate, 0.05 μM β-mercaptoethanol, and 50 μg/ml penicillin/streptomycin. The filtrated supernatant was used as WEHI-3 conditioned medium.

After 16-h culture, the nonadherent bone marrow cells were transferred to a new flask for further culture. The medium was refreshed once a week. After 4 weeks, the cells were analyzed by flow cytometry

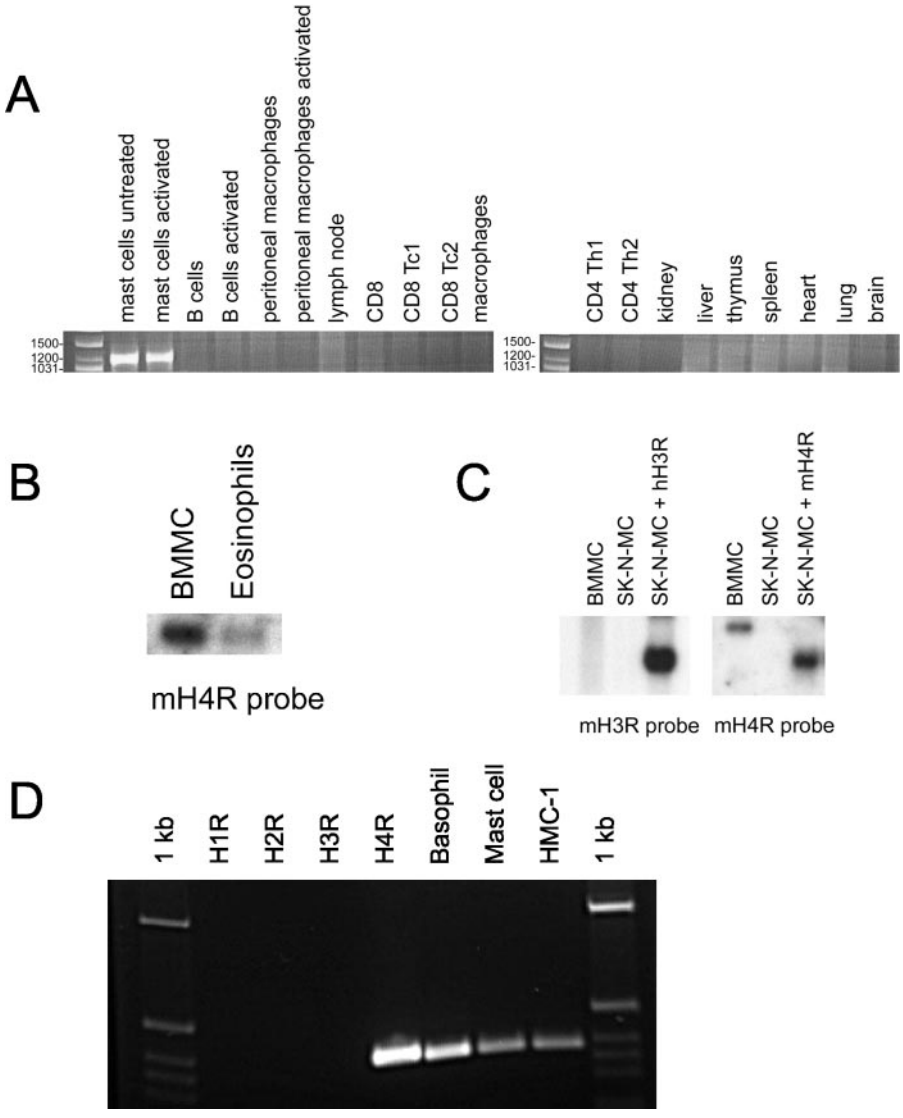

Fig. 2. Mouse H_4 receptor expression is restricted to mast cells, basophils, and eosinophils. A, cDNA of different mouse tissues and cell types were used as templates for reverse transcription-PCR. The expected PCR product for mouse H_4 receptor is 1185 bp. Mast cells were primed with antigen-specific IgE (untreated) and activated with antigens for 30 min. B cells and macrophages were activated with lipopolysaccharide for 24 and 1 h, respectively. B, Northern blot analysis of mouse mast cells and eosinophils. The blot was probed with the mouse H_4 receptor. C, Northern blots analysis of mouse mast cells (bone marrow mast cells, BMMC) for H_3 and H_4 receptor expression. In the left blot mast cells, human (h) H_3 receptor transfected and untransfected SK-N-MC were blotted with a mouse H_3 receptor probe. In the right blot, mast cells, mouse H_4 receptor transfected and untransfected SK-N-MC were blotted with a mouse H_4 receptor probe. The difference in size in transfected and endogenous receptor is due to the presence of the untranslated regions in endogenous H_4 receptor RNA, which are deleted in the transfected receptor. D, RT-PCR detection of human H_4 receptor expression in cDNA from human basophils, cord blood-derived mast cells, and HMC-1 cells. SK-N-MC cells transfected with human H_1, H_2, H_3, and H_4 receptors were used as controls. The expected size of the human H_4 receptor PCR product is 350 bp.

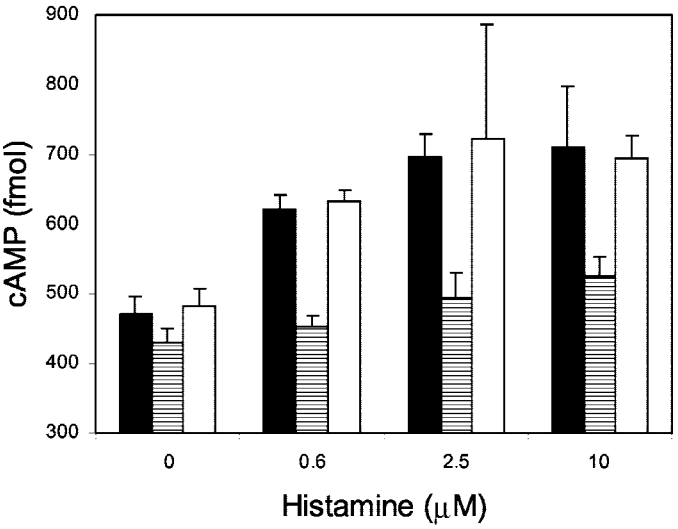

Fig. 3. Histamine induces cyclic AMP in mast cells through H_2 receptors, but not H_4 receptors. Mast cells of $H_3R-/-$ mice were activated with vehicle (black columns), 10 μM ranitidine (striped columns), or 10 μM thioperamide (white columns) followed by 0.6, 2.5, or 10 μM histamine. Shown are average values ± standard deviations of triplicate determinations. The experiment was repeated three times on different batches of cells.

for IgE receptor and CD117 (c-kit), which are expressed specifically on mast cells. IgE receptor on mast cells were detected by incubating with anti-DNP IgE or vehicle for 30 min, followed by fluorescein isothiocyanate-labeled anti-IgE antibody. Mast cells were incubated with fluorescein isothiocyanate-labeled anti-CD117 antibody for 30 min on ice. The majority of the bone marrow cells was confirmed to be mast cells with >99% IgE receptor positive and >99% CD117 positive. Mast cells of 4 to 8 weeks were used for experiments. No difference in proliferation and in expression of IgE receptor or c-kit receptor was observed in mast cells derived from $H_3R-/-$, $H_4R-/-$, and wild-type mice.

Degranulation Assay. Mast cells (5×10^5/ml) were sensitized overnight with 2 μg/ml anti-DNP IgE. Mast cells (2×10^5/well) were plated out in a 96-wells plate and incubated for 15 min with 10 μM histamine or vehicle at 37°C. Degranulation was achieved by adding different concentrations of DNP-HSA for an additional 30 min. Total release of mast cell contents was achieved by adding 1% Triton X-100. The plates were spun down (1000 rpm, 5 min, 5°C), and supernatants were analyzed for β-hexosaminidase.

β-Hexosaminidase was measured by adding 25 μl of supernatant to 50 μl of 10 mM *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide in 0.1 M sodium citrate buffer (pH 4.5) for 2 h at 37°C. The reaction was stopped by adding 50 μl of 0.4 M glycine (pH 9). The plates were measured at wavelength 405 nm. The percentage of degranulation was calculated as $((A - B)/(T - B) \times 100)$, where A is levels of β-hexosaminidase released from stimulated cells, B is that released from unstimulated cells, and T is total content of the cells.

To study the induction of leukotriene and prostaglandins, IgE-sensitized mast cells (1×10^6/well) were incubated for 15 min with 10 μM thioperamide or vehicle, followed by 30- or 210-min incubation with 10 μM histamine or 5 μg/ml compound 48/80. LTB_4 and prostaglandin levels were measured in supernatants according to the manufacturer's instruction.

Chemotaxis Assay. Transwells with a pore size of 8 μm were coated with 100 μl of 100 ng/ml bovine fibronectin for 2 h at room temperature. After removal of the fibronectin, 600 μl of RPMI 1640 medium with 1% BSA in the presence of histamine (ranging from 1.25 to 20 μM) was added to the bottom chamber. Subsequently, 10 μM histamine receptor antagonists (diphenhydramine, ranitidine, thioperamide), U73122 (1.1, 3.3, and 10 μM), or U73433 (1.1, 3.3 and 10 μM) was added to the top and bottom chambers. Mast cells (2×10^5/well) were added to the top chamber. The plates were incubated for 3 h at 37°C. Transwells were removed, and the number of cells in the bottom chamber was counted for 1 min by flow cytometer. To study PTX effects, mast cells (1×10^6 cells/ml) were pretreated for 16 h with 0, 0.5, 5, or 50 ng/ml PTX. Cells were washed afterward and put in the upper chamber as described above.

Calcium Mobilization. Mast cells (2×10^5/well) were loaded with 4 μM calcium dye Fluo-3 (acetoxymethyl ester) in dye-loading buffer for 1 h at 37°C. The dye-loading buffer is RPMI 1640 medium without phenol red and contains 0.5% BSA, 2.5 mM probenecid, and 0.08% Pluronic acid. Cells were spun down and taken up in loading medium which is RPMI 1640 medium (without phenol red) containing 0.5% BSA. Cells were plated out in polylysine-coated black wall 96-well tissue culture plates. Before measurements, the plates were spun for 3 min at 1000 rpm at room temperature. Calcium mobilization was assayed in a fluorometric imaging plate reader 384 (Molecular Devices Corp., Sunnyvale, CA). The fluorescence intensity was calculated as the maximum minus the minimum fluorescence over a 2-min period. All data points were done in triplicates, and experiments were repeated at least three times with different batches of mast cells.

Histamine receptor agonists and antagonists were added to the cells 10 min before the calcium measurements. In calcium storage experiments, Fluo-3-loaded mast cells received a first addition of 3 mM EDTA or 10 μM thapsigargin or PBS. After stabilization of the signal, mast cells received a second addition of 10 μM histamine.

For PTX treatment, mast cells (1×10^6 cells/ml) were pretreated for 16 h with 0, 0.5, 5, or 50 ng/ml PTX. Cells were washed and loaded with Fluo-3 as described above. For phospholipase C (PLC) inhibitor treatment, mast cells were treated with 1.1, 3.3, and 10 μM U73122 or U73433 10 min before stimulation with 10 μM histamine.

To detect calcium response triggered by IgE receptor cross-linking, mast cells (5×10^5/ml) were sensitized overnight with 2 μg/ml anti-DNP IgE. Cells were washed and loaded with Fluo-3 as described above. During the calcium measurements, 5 μM histamine was added followed 3 min later with different concentrations of DNP-HSA.

cAMP Measurements. Mast cells (1×10^6/well) in culture medium containing 1 μM 3-isobutyl-1-methylxanthine were plated out in a 96-well plate. Cells were incubated for 30 min at 37°C. Histamine receptor antagonist (10 μM) was added 15 min before histamine and/or 100 μM forskolin addition for 30 min at 37°C. Intracellular cAMP levels in cell lysates were determined using the Biotrack cAMP enzyme immunoassay system according to the manufacturer's instructions.

Results

Mast Cells, Basophils, and Eosinophils Express H_4 Receptors. Previously, we reported that the human H_4 receptor is expressed mainly in bone marrow and eosinophils (Liu et al., 2001a). In the present study, mouse H_4 expression was determined in different tissues and purified cell types of the hemopoietic lineage. Abundant expression of mouse H_4 receptor was detected in untreated mast cells and antigen-activated IgE-primed mast cells by RT-PCR (Fig. 2A). In contrast, H_4 receptor was not detected in any of the tissues tested, such as lymph nodes, kidney, liver, thymus, spleen, heart, lung, and brain. In addition, expression was not detected in many different immune cell types, including $CD4^+$ effector Th1 and Th2 cells, $CD8^+$ effector Tc1 and Tc2 cells, resting and LPS-activated B cells, as well as macrophages.

Expression of H_4 receptor in mast cells and eosinophils was further confirmed by Northern blot analysis (Fig. 2, B and C). Both H_1 and H_2 receptors were detected on mast cells (data not shown), whereas H_3 receptor was undetectable by North-

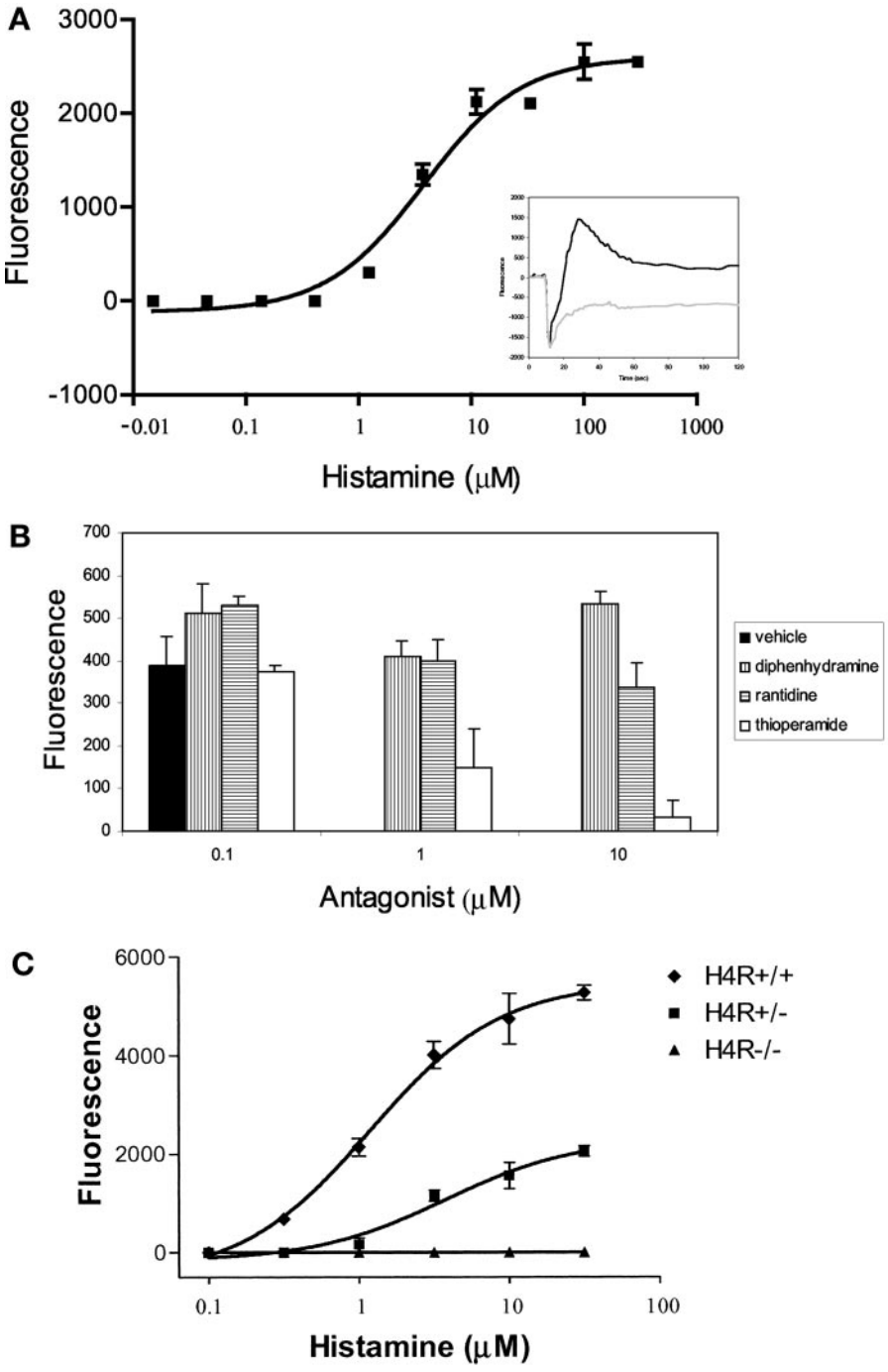

Fig. 4. Histamine induces calcium mobilization in mast cells through H_4 receptors. A, histamine induces calcium mobilization in mast cells in a concentration-dependent manner. A typical tracing (insert) of control treatment (light gray tracing) and 20 μM histamine (black tracing) and concentration-response curve are shown. The decrease in fluorescent signal upon addition of histamine was due to a disturbance of the signal by the pipetting system of the machine. When nonadherent cells such as mast cells are used, addition of reagents may slightly stir up cells and therefore decrease the fluorescent signal transiently. B, mast cells from $H_3R-/-$ mice were treated with vehicle (black columns), or various concentrations of diphenhydramine (H_1 receptor antagonist, vertical striped columns), ranitidine (H_2 receptor antagonist, horizontal striped columns) or thioperamide (H_3/H_4 antagonist, white columns) followed with 10 μM histamine. C, concentration-response curve of histamine-induced calcium mobilization using mast cells from wild-type (diamonds), $H_4R+/-$ (squares), and $H_4R-/-$ mice (triangles). Calcium mobilization was determined using fluorometric imaging plate reader, and fluorescence intensity was calculated as the maximum minus the minimum fluorescence over a 2-min period. Shown are average values ± standard deviations of triplicate determinations. These graphs are representative of at least three similar experiments.

ern blot analysis (Fig. 2C, left) and RT-PCR (data not shown). Consistent with the expression profile in mice, H_4 receptor was detected in human cord blood-derived mast cells and in human HMC-1 mast cell line by RT-PCR (Fig. 2D). In addition, human H4 receptor was expressed in basophils (Fig. 2D) but not in neutrophils (data not shown). In summary, our data show that the H_4 receptor is expressed on mast cells, basophils, and eosinophils.

H_4 Receptors Mediate Calcium Mobilization in Mast Cells. Histamine binding to its receptors activate G proteins, which result in changes of calcium or cAMP levels. Histamine induces a concentration-dependent increase of cAMP in mast cells (Fig. 3). This response was unaffected by H_3/H_4 antagonist thioperamide and H_1 antagonist diphenhydramine (data not shown), thereby excluding a role for H_1, H_3, or H_4 receptors. However, the H_2 receptor antagonist ranitidine could inhibit the histamine-induced cAMP increase. The results indicate that the histamine-induced cAMP increase in mast cells is H_2 receptor-mediated.

Calcium mobilization was observed in mast cells induced by histamine in a concentration-dependent manner (Fig. 4A). The response peaked at about 20 s after histamine addition and returned to basal levels within 1 min (Fig. 4A, inset). The ED_{50} value of histamine-induced calcium mobilization was 3.8 μM. Neither H_1 receptor antagonists nor H_2 receptor antagonists altered the histamine-induced calcium mobiliza-

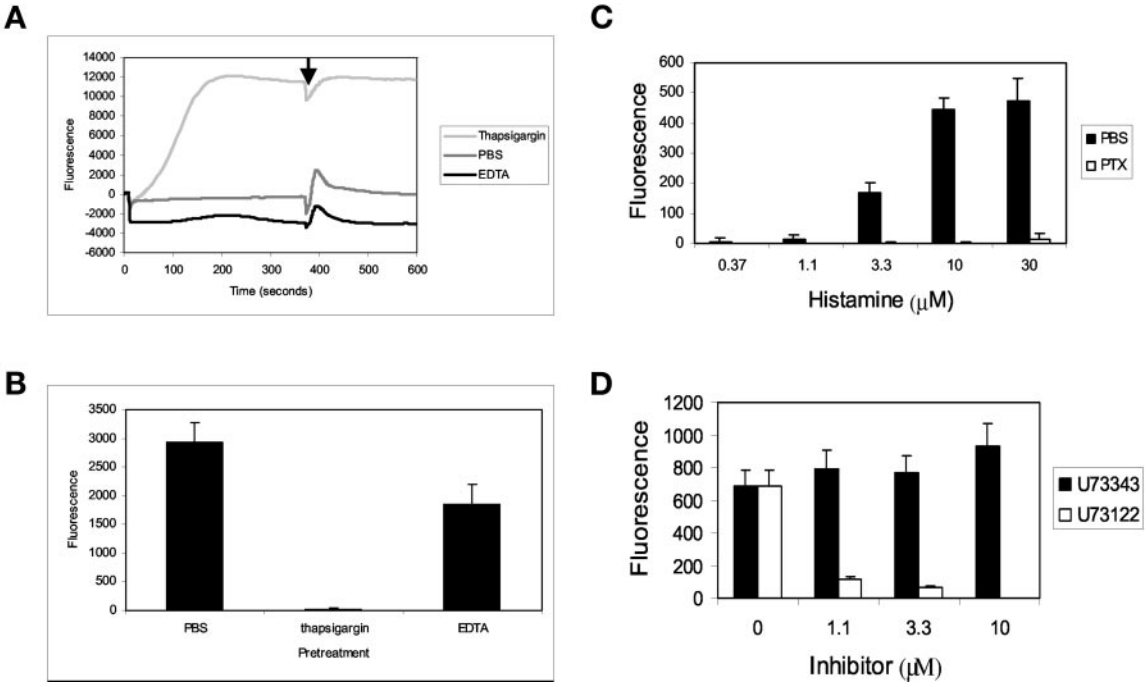

Fig. 5. Histamine-induced calcium mobilization through the H_4 receptor is derived from intracellular stores and is mediated through PLC- and PTX-sensitive pathways. A and B, mast cells were treated at t = 10 s with 10 μM thapsigargin, 3 mM EGTA or PBS, followed by 10 μM histamine at t = 370 s (arrow). A, typical tracing (light gray, thapsigargin; dark gray, PBS; black, EDTA). B, peak values of histamine-induced calcium mobilization after PBS, thapsigargin, or EDTA pretreatment. C, mast cells were treated for 16 h with PBS (black columns) or 50 ng/ml PTX (white columns), followed by a concentration-response curve of histamine. D, mast cells were treated for 10 min with U73122 (white columns) or U73343 (black columns) before stimulation with 10 μM histamine. Calcium mobilization was determined using fluorometric imaging plate reader, and fluorescence intensity was calculated as the maximum minus the minimum fluorescence over a 2-min period. Shown are average values ± standard deviations of triplicate determinations. These graphs are representative of at least three similar experiments.

tion (Fig. 4B). However, thioperamide (Fig. 4B) inhibited the histamine-induced calcium mobilization in a concentration-dependent manner, with an IC_{50} value of 1.00 ± 0.5 μM. This IC_{50} value is consistent with the relative binding affinities of histamine and thioperamide (Liu et al., 2001a). Together, the data suggest that H_3 and/or H_4 receptors are involved in calcium mobilization in mast cells.

Because mast cells do not express H_3 receptors (Fig. 2C), it is likely that the calcium response is mediated by H_4 receptors. A direct proof of H_4 receptor-mediated calcium mobilization was demonstrated in mast cells generated from $H_4R-/-$ and $H_3R-/-$ mice. In contrast to wild-type mast cells, up to 30 μM histamine stimulation in $H_4R-/-$ mast cells did not result in calcium mobilization (Fig. 4C). Mast cells from $H_4R+/-$ mice showed an intermediate calcium response compared with mast cells from wild-type mice. This response is histamine-specific because $H_4R-/-$ mast cells mediated normal calcium responses to ATP or ionomycin (data not shown). Furthermore, $H_3R-/-$ mast cells showed a normal calcium response to histamine comparable with that in wild-type mast cells (data not shown). Therefore, it can be concluded that histamine induces calcium mobilization in mast cells via the H_4 receptor.

H_4 Receptors Trigger Calcium Release from Intracellular Calcium Stores. To determine the source of calcium in histamine-induced calcium mobilization, either EDTA or thapsigargin was used in experiments to deplete calcium from extracellular environment or intracellular calcium storage, respectively. Histamine-induced calcium mobilization was not affected by EDTA but was completely abolished by thapsigargin (Fig. 5, A and B). Thus, histamine mediates the release of calcium from intracellular calcium stores in mast cells.

H_4 Receptor Mediates Calcium Mobilization through Gαi/o Proteins and PLC. To determine the G proteins used by H4 receptor in mast cells, Gαi/o protein inhibitor PTX was used in experiments. Pretreatment of mast cells with PTX inhibited the histamine-induced calcium response completely (Fig. 5C), but the calcium response toward ionomycin or ATP was unaffected (data not shown), indicating that the PTX inhibitory effect is histamine-specific. Therefore, it seems that Gαi/o proteins are acting downstream of the H_4 receptor, leading to calcium mobilization.

The possible involvement of PLC in histamine-induced calcium mobilization was studied using the PLC inhibitor U73122 and its inactive analog U73343 (Thompson et al., 1991). U73122 inhibited the histamine-induced calcium mobilization in a concentration-dependent manner with a complete inhibition at 10 μM, whereas the inactive analog U73343 (up to 10 μM) was unable to alter this response (Fig. 5D). These results indicate that the histamine effects on calcium mobilization involved PLC activation.

Histamine Does Not Alter Degranulation through H_4 Receptors. Effects of histamine on IgE receptor-mediated calcium response and degranulation in mast cells were investigated. IgE-primed mast cells were pretreated with histamine, followed by antigen stimulation. Histamine did not alter antigen-IgE triggered calcium mobilization (Fig. 6A). Antigen induced degranulation of IgE-primed mast cells from wild-type and $H_4R-/-$ mice was also unaffected by hista-

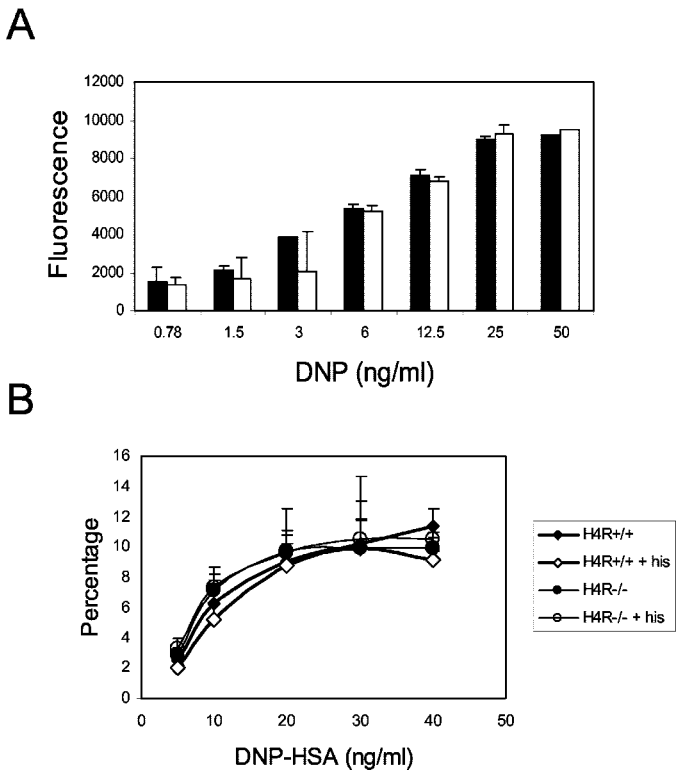

Fig. 6. Histamine does not induce mast cell degranulation or alter antigen-induced degranulation. A, mast cells incubated overnight with 5 μg/ml IgE and were treated with vehicle (white columns) or 5 μM histamine (black columns) followed 3 min later with different concentrations of the antigen DNP-HSA. Calcium mobilization was determined using a fluorometric imaging plate reader. B, mast cells were incubated overnight with 5 μg/ml IgE. Mast cells from $H_4R-/-$ (diamonds) and wild-type (circles) mice were incubated for 15 min with vehicle (black) or 10 μM histamine (white) followed by 30-min incubation with DNP-HSA. In the supernatants, levels of β-hexosaminidase were determined.

mine pretreatment (Fig. 6B). In addition, thioperamide did not have any effects on antigen-IgE-mediated mast cell degranulation (data not shown).

The effects of histamine on the production of de novo-synthesized mediators such as prostaglandins and leukotrienes were also investigated. Compound 48/80 induced LTB_4 and prostaglandin release by mast cells, whereas histamine did not alter LTB_4 or prostaglandin levels (Table 1). In summary, H_4 receptor and histamine do not seem to be involved in antigen-induced degranulation because histamine does not induce degranulation nor is it involved in the de novo production of LTB_4 and prostaglandins by mast cells.

Histamine Mediates Chemotaxis through H_4 Receptors. Chemotaxis of mast cells toward histamine was investigated using a Transwell system. Histamine induced mast cell migration in a concentration-dependent manner (Fig. 7A). This observed effect was due to chemotaxis but not chemokinesis, because cell migration was abolished when the histamine concentration gradient was disrupted. Thioperamide inhibited histamine-induced mast cells chemotaxis in a concentration-dependent manner, whereas neither diphenhydramine nor ranitidine had any effects (Fig. 7, B and C). The IC_{50} value of thioperamide in chemotaxis is similar to that in the calcium mobilization assay. To distinguish between H_3 and H_4 receptor-mediated effects on chemotaxis of mast cells, the chemotaxis assay was performed using mast cells derived from $H_4R-/-$ or $H_3R-/-$ mice. No migration of $H_4R-/-$ mast cells toward histamine was observed (Fig. 7A). In contrast, chemotaxis of $H_3R-/-$ mast cells to histamine was similar to that in wild-type mast cells (data not shown). Thus, histamine-induced chemotaxis of mast cells is mediated through the H_4 receptor.

H_4 Receptor-Mediated Chemotaxis Involves Gαi/o Proteins and PLC. Similar to the PTX inhibitory effects on histamine-induced calcium mobilization, preincubation of mast cells with PTX caused a concentration-dependent decrease in mast cell chemotaxis toward histamine (Fig. 7D). A complete inhibition of histamine-induced chemotaxis was observed at 50 ng/ml, a concentration with similar effects in inhibiting calcium mobilization. PLC was also involved in the histamine-induced chemotaxis because U73122 could inhibit the chemotaxis in a concentration dependent fashion (Fig. 7E), while its inactive analog U73343 did not alter the chemotaxis. Together, these results suggest that the histamine-induced chemotaxis of mast cells involves Gαi/o proteins and PLC, similar to that of the calcium response.

Discussion

In the present study, we showed that the mouse H_4 receptor is expressed on mast cells and eosinophils but not on other hemopoietic cells, including T cells, B cells, or macrophages. Furthermore, human H_4 receptor is expressed on mast cells and basophils in addition to the previously reported expression on human eosinophils (Oda et al., 2000; Liu et al., 2001a; Morse et al., 2001). Interestingly, this is the first study to show that mast cells express the H_4 receptor, but not the H_3 receptor. In the literature, it has been unclear whether mast cells express the H_3 receptor. Most studies used thioperamide as a specific H_3 antagonist (Kohno et al., 1994; Bissonnette, 1996), but recent data indicate that both the mouse and human H_4 receptor can bind thioperamide as well (Liu et al., 2001b). It is therefore likely that the effects of histamine on mast cells that can be blocked by thioperamide are actually mediated by H_4 receptors.

The role of the H_4 receptor in some of the physiological functions of mast cells was investigated. One of the major

TABLE 1

Mast cell production of prostaglandin and LTB_4

Mast cells were incubated with anti-DNP IgE for 16 h followed by 15-min incubation with 10 μM thioperamide, 30- or 210-min incubation with 10 μM histamine, or 5 μg/ml compound 48/80. Maximal was measured by lysing the cells with 1% Triton X-100. Prostaglandin and LTB4 levels in the supernatants were measured.

	T	Maximum	Untreated	Histamine	Histamine + Thioperamide	Compound 48/80
	min					
Prostaglandin	30	810 ± 16	28 ± 3	33 ± 0.3	34 ± 3	51 ± 6
	210	789 ± 98	50 ± 13	44 ± 5	37 ± 0.3	73 ± 5
LTB_4	30	56 ± 2	5 ± 0.7	6 ± 1.6	5 ± 1.5	53 ± 14
	210	58 ± 4	10 ± 3	8 ± 2	7 ± 0.5	107 ± 37

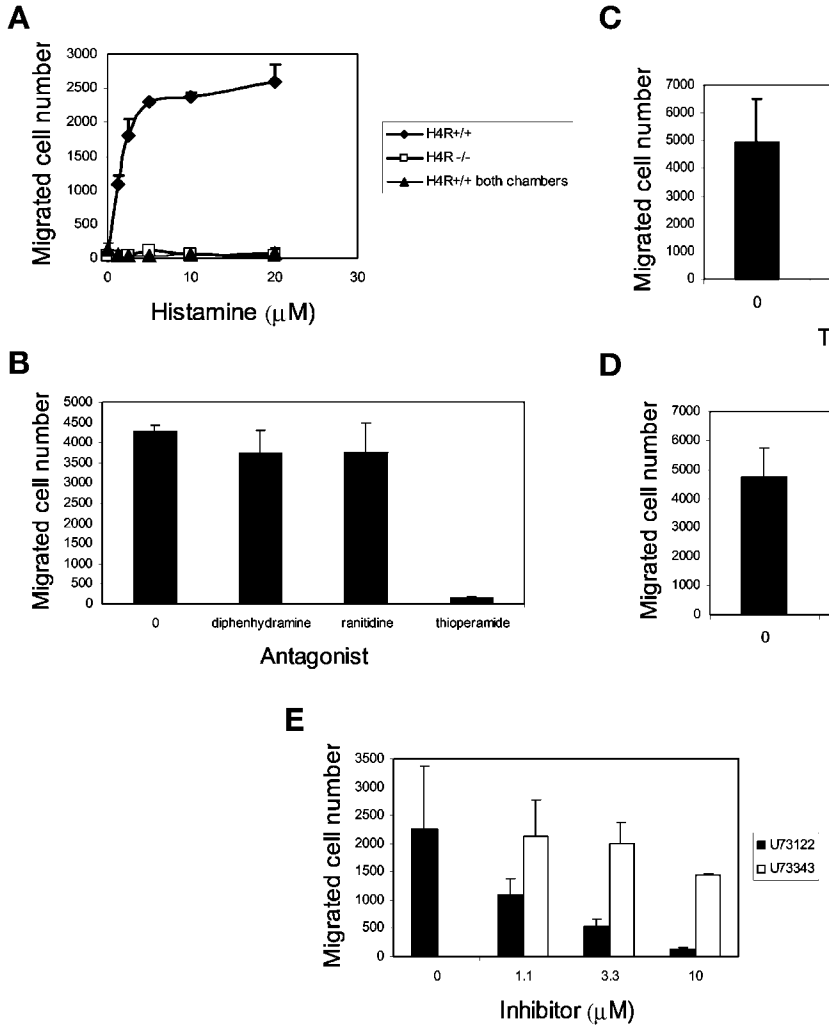

Fig. 7. Histamine induces chemotaxis of mast cells through H_4 receptors. A, chemotaxis of wild-type (black diamonds) and $H_4R-/-$ (white squares) mast cells to different concentrations of histamine. As a negative control, wild-type mast cells were added to the upper chamber, while histamine was added to both chambers (black triangles). B, in the lower chamber, 10 μM histamine was added, while in both chambers 10 μM histamine receptor antagonists were added and mast cells were added in the upper chamber. C, in the lower chamber, 10 μM histamine was added, while in both chambers thioperamide were added and mast cells were added in the upper chamber. D, mast cells were treated for 16 h with pertussis toxin. In the lower chamber, 10 μM histamine was added and mast cells were added to the upper chamber. E, in the lower chamber, 10 μM histamine was added, while in both chambers 10 μM U73122 (black columns) or 10 μM U73343 (white columns) was added and mast cells were added to the upper chamber. Each experiment was performed at least in triplicate.

biological functions of mast cells is to release inflammatory mediators in response to antigens. The major mechanism of such release is through IgE-mediated degranulation. The present work shows that histamine does not seem to have any effects on degranulation, either on its own or in combination with antigen-IgE complexes. In addition, histamine does not seem to alter mast cell proliferation or survival (data not shown). Similarly, $H_4R-/-$ mast cells did not show any defects in degranulation, proliferation, or survival, indicating that the H_4 receptor has no role in these processes.

Mast cell progenitor cells, which are present in the bone marrow, migrate to connective or mucosal tissue where they differentiate into the mature form. It is thought that chemoattractants such as stem cell factor might be important for this localization. Migration of mast cells may also play a role in allergic rhinitis and allergy where increases in mast cell number are found (Kirby et al., 1987; Crimi et al., 1991; Amin et al., 2000; Gauvreau et al., 2000; Kassel et al., 2001). In addition, it is known that in response to antigens there is a redistribution of mast cells to the epithelial lining of the nasal mucosa (Fokkens et al., 1992; Slater et al., 1996). It is possible that some of the redistribution that is seen in allergic conditions may be mediated by histamine because it would be continually produced under such circumstances. The data presented here show that histamine is a potent chemoattractant for mast cells and that this chemotaxis is mediated via the H_4 receptor. Antagonists of the H_4 receptor may therefore be useful in the treatment of asthma or allergic rhinitis. Currently, we are addressing these questions with in vivo models.

Using specific histamine receptor antagonists as well as mast cells derived from $H_4R-/-$ and $H_3R-/-$ mice, we demonstrated that histamine induced calcium mobilization from intracellular stores in mast cells via the H_4 receptor. Calcium mobilization via the H_4 receptor has also been observed using cells cotransfected with both the H_4 receptor and chimeric G proteins (Morse et al., 2001). However, other studies using human H_4 receptor-transfected cells have shown that hista-

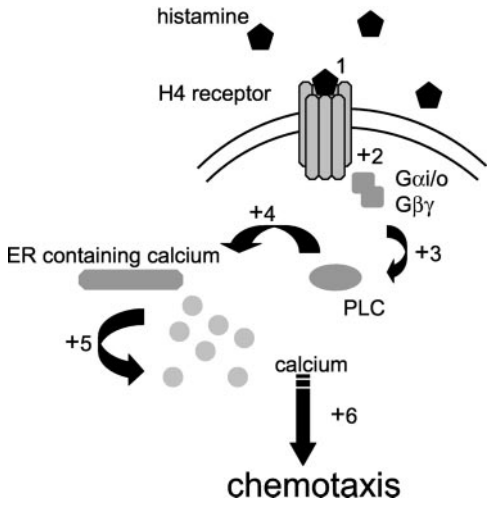

Fig. 8. Signaling pathway of H_4 receptor on mast cells. Histamine binds to the receptor (1), which will activate Gαi/o proteins (2). Activation of G proteins will activate PLC (3), which hydrolyzes IP_3. IP_3 possibly activates IP_3 receptors on the endoplasmic reticulum (ER, 4), causing the release of intracellular calcium (5). Via unknown pathways, this will lead to chemotaxis (6).

mine activation of the cells resulted mainly in decreased cAMP levels (Oda et al., 2000; Liu et al., 2001a; Zhu et al., 2001). Because results of transfected cells depend on the endogenous machinery of the cell, different cell types can yield different results. In the present study, these complications are not present, because the endogenous H_4 receptor was studied. Previously, similar calcium mobilization induced by histamine was demonstrated in human eosinophils, which have been shown to express the H_4 receptor (Raible et al., 1994). The calcium mobilization was inhibited by thioperamide, the dual H_3/H_4 receptor antagonist. Nevertheless, *R*-α-methyl-histamine and *N*-α-methyl-histamine were less potent than histamine in inducing calcium mobilization, which is more consistent with their respective affinities for the H_4 receptor than for the H_3 receptor (Raible et al., 1994). Therefore, it is likely that this response is mediated by the H_4 receptor and not the H_3 receptor. Thus, the activation of H_4 receptor both on mast cells and eosinophils results in calcium mobilization.

The signaling pathways activated by the H_4 receptor have also been studied. Both Gαi/o proteins and PLC are involved because PTX, which inactivates Gαi/o proteins, and the PLC inhibitor U73122, inhibited chemotaxis and calcium mobilization. Gαi/o proteins do not activate PLCβ, but G protein βγ subunits can activate $PLC\beta_{2/3}$ (Exton, 1996; Clapham and Neer, 1997; Rhee, 2001). It is therefore possible that $PLC\beta_{2/3}$ is activated by the G protein βγ subunits that are dissociated from Gαi/o proteins when histamine binds to the H_4 receptor. Other G protein-coupled receptors have also been shown to signal via PLC and Gαi/o (Seebeck et al., 1998; Zussman et al., 1998; Yang et al., 2002). The activation of PLC may lead to the release of inositol-1,4,5-triphosphate (IP_3). IP_3 can activate an IP_3 receptor in the endoplasmic reticulum, which causes the release of calcium in the cytoplasm, a mechanism that is known to occur in mast cells (Pacher et al., 2000). Compound 48/80 has been reported to elicit calcium response in mast cells through Gαi/o proteins, phosphatidylinositol 3-kinase, Src, and Syk (Shefler and Sagi-Eisenberg, 2001). Stem cell factor is also known to induce calcium mobilization in mast cells involving activation of Gαi/o proteins, phosphatidylinositol 3-kinase, p38 mitogen-activated protein, and mitogen-activated protein kinase kinases (Dastych et al., 1998; Sundstrom et al., 2001).

We propose the following signaling pathway involved in histamine activation of the H_4 receptor (Fig. 8). Histamine binds to the H_4 receptor on mast cells and eosinophils and causes the activation of PTX-sensitive Gαi/o proteins. Possibly G protein βγ subunits dissociated from Gαi/o proteins trigger the activation of PLC. PLC hydrolyzes phosphatidylinositol 4,5-biphosphate to diacylglycerol and IP_3. IP_3 activates a calcium channel in the endoplasmic reticulum, possibly through an IP_3 receptor to release calcium. The increased calcium levels trigger currently unknown signaling pathways, which will cause mast cell chemotaxis toward histamine.

Acknowledgments

We thank Karen Ngo for technical help in genotyping H_4 receptor-deficient mice and in culturing bone marrow derived mast cells. We also thank Dr. Tim Lovenberg for providing the human SK-N-MC cells transfected with different histamine receptors.

References

Amin K, Ludviksdottir D, Janson C, Nettelbladt O, Bjornsson E, Roomans GM, Boman G, Seveus L, and Venge P (2000) Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. *Am J Respir Crit Care Med* **162:**2295–2301.

Bissonnette EY (1996) Histamine inhibits tumor necrosis factor α release by mast cells through H2 and H3 receptors. *Am J Respir Cell Mol Biol* **14:**620–626.

Clapham DE and Neer EJ (1997) G protein βγ subunits. *Annu Rev Pharmacol Toxicol* **37:**167–203.

Crimi E, Chiaramondia M, Milanese M, Rossi GA, and Brusasco V (1991) Increased numbers of mast cells in bronchial mucosa after the late-phase asthmatic response to allergen. *Am Rev Respir Dis* **144:**1282–1286.

Dahl C, Saito H, Nielsen HV, and Schiotz PO (2002) The establishment of a combined serum-free and serum-supplemented culture method of obtaining functional cord blood-derived human mast cells. *J Immunol Methods* **262:**137–143.

Dastych J, Taub D, Hardison MC, and Metcalfe DD (1998) Tyrosine kinase-deficient Wv c-kit induces mast cell adhesion and chemotaxis. *Am J Physiol* **275:**C1291–C1299.

Exton JH (1996) Regulation of phosphoinositide phospholipases by hormones, neurotransmitters and other agonists linked to G proteins. *Annu Rev Pharmacol Toxicol* **36:**481–509.

Fokkens WJ, Godthelp T, Holm AF, Blom H, Mulder PG, Vroom TM, and Rijntjes E (1992) Dynamics of mast cells in the nasal mucosa of patients with allergic rhinitis and non-allergic controls: a biopsy study. *Clin Exp Allergy* **22:**701–710.

Gauvreau GM, Lee JM, Watson RM, Irani AM, Schwartz LB, and O'Byrne PM (2000) Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatics. *Am J Respir Crit Care Med* **161:**1473–1478.

Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, Schunack W, Levi R, and Haas HL (1997) International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol Rev* **49:**253–278.

Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OA, Malolepszy J, Zak-Nejmark T, Koga R, Kobayashi T, Blaser K, et al. (2001) Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature (Lond)* **413:**420–425.

Kassel O, de Blay F, Duvernelle C, Olgart C, Israel-Biet D, Krieger P, Moreau L, Muller C, Pauli G, and Frossard N (2001) Local increase in the number of mast cells and expression of nerve growth factor in the bronchus of asthmatic patients after repeated inhalation of allergen at low-dose. *Clin Exp Allergy* **31:**1432–1440.

Kirby JG, Hargreave FE, Gleich GJ, and O'Byrne PM (1987) Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis* **136:**379–383.

Kohno S, Nakao S, Ogawa K, Yamamura H, Nabe T, and Ohata K (1994) Possible participation of histamine H3-receptors in the regulation of anaphylactic histamine release from isolated rat peritoneal mast cells. *Jpn J Pharmacol* **66:**173–180.

Leurs R, Smit MJ, and Timmerman H (1995) Molecular pharmacological aspects of histamine receptors. *Pharmacol Ther* **66:**413–463.

Liu C, Ma X, Jiang X, Wilson SJ, Hofstra CL, Blevitt J, Pyati J, Li X, Chai W, Carruthers N, and Lovenberg TW (2001a) Cloning and pharmacological characterization of a fourth histamine receptor (H_4) expressed in bone marrow. *Mol Pharmacol* **59:**420–426.

Liu C, Wilson SJ, Kuei C, and Lovenberg TW (2001b) Comparison of human, mouse, rat and guinea pig histamine H4 receptors reveals substantial pharmacological species variation. *J Pharmacol Exp Ther* **299:**121–130.

Lovenberg TW, Pyati J, Chang H, Wilson SJ, and Erlander MG (2000) Cloning of rat histamine H_3 receptor reveals distinct species pharmacological profiles. *J Pharmacol Exp Ther* **293:**771–778.

Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, Jackson MR, and

Erlander MG (1999) Cloning and functional expression of the human histamine H3 receptor. *Mol Pharmacol* **55:**1101–1107.
Morse KL, Behan J, Laz TM, West RE Jr, Greenfeder SA, Anthes JC, Umland S, Wan Y, Hipkin RW, Gonsiorek W, et al. (2001) Cloning and characterization of a novel human histamine receptor. *J Pharmacol Exp Ther* **296:**1058–1066.
Nguyen T, Shapiro DA, George SR, Setola V, Lee DK, Cheng R, Rauser L, Lee SP, Lynch KR, Roth BL, and O'Dowd BF (2001) Discovery of a novel member of the histamine receptor family. *Mol Pharmacol* **59:**427–433.
Oda T, Morikawa N, Saito Y, Masuho Y, and Matsumoto S (2000) Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J Biol Chem* **275:**36781–36786.
Pacher P, Csordas P, Schneider T, and Hajnoczky G (2000) Quantification of calcium signal transmission from sarco-endoplasmic reticulum to the mitochondria. *J Physiol (Lond)* **529:**553–564.
Raible DG, Lenahan T, Fayvilevich Y, Kosinski R, and Schulman ES (1994) Pharmacologic characterization of a novel histamine receptor on human eosinophils. *Am J Respir Crit Care Med* **149:**1506–1511.
Rhee SG (2001) Regulation of phosphoinositide-specific phospholipase C. *Annu Rev Biochem* **70:**281–312.
Seebeck J, Kruse ML, Schmidt-Choudhury A, and Schmidt WE (1998) Pituitary adenylate cyclase activating polypeptide induces degranulation of rat peritoneal mast cells via high-affinity PACAP receptor-independent activation of G proteins. *Ann NY Acad Sci* **865:**141–146.
Shefler I and Sagi-Eisenberg R (2001) Gi-mediated activation of the Syk kinase by the receptor mimetic basic secretagogues of mast cells: role in mediating arachidonic acid/metabolites release. *J Immunol* **167:**475–481.
Shier P, Hofstra CL, Ma XJ, Wu Y, Ngo K, and Fung-Leung WP (2000) Tbt-1, a new T-box transcription factor induced in activated Th1 and CD8+ T cells. *Immunogenetics* **51:**771–778.
Slater A, Smallman LA, and Drake-Lee AB (1996) Increase in epithelial mast cell numbers in the nasal mucosa of patients with perennial allergic rhinitis. *J Laryngol Otol* **110:**929–933.
Sundstrom M, Alfredsson J, Olsson N, and Nilsson G (2001) Stem cell factor-induced migration of mast cells requires p38 mitogen- activated protein kinase activity. *Exp Cell Res* **267:**144–151.
Szeberenyi JB, Pallinger E, Zsinko M, Pos Z, Rothe G, Orso E, Szeberenyi S, Schmitz G, Falus A, and Laszlo V (2001) Inhibition of effects of endogenously synthesized histamine disturbs in vitro human dendritic cell differentiation. *Immunol Lett* **76:**175–182.
Thompson AK, Mostafapour SP, Denlinger LC, Bleasdale JE, and Fisher SK (1991) The aminosteroid U-73122 inhibits muscarinic receptor sequestration and phosphoinositide hydrolysis in SK-N-SH neuroblastoma cells. A role for Gp in receptor compartmentation. *J Biol Chem* **266:**23856–23862.
Toyota H, Dugovic C, Koehl M, Laposky AD, Weber C, Ngo K, Wu Y, Lee DH, Yanai K, Sakurai E, et al. (2002) Behavioral characterization of mice lacking histamine H3 receptors. *Mol Pharmacol* **62:**389–397.
Yang C-M, Hsiao L-D, Chien C-S, Lin C-C, Luo S-F, and Wang C-C (2002) Substance P-induced activation of p42/44 mitogen-activated protein kinase associated with cell proliferation in human tracheal smooth muscle cells. *Cell Signal* **14:**913–923.
Zhu Y, Michalovich D, Wu H, Tan KB, Dytko GM, Mannan IJ, Boyce R, Alston J, Tierney LA, Li X, et al. (2001) Cloning, expression and pharmacological characterization of a novel human histamine receptor. *Mol Pharmacol* **59:**434–441.
Zussman A, Hermuet S, and Sagi-Eisenberg R (1998) Stimulation of Ca^{2+}-dependent exocytosis and arachidonic acid release in cultured mast cells (RBL-2H3) by a GTPase-deficient mutant of Gαi3. *Eur J Biochem* **258:**144–149.

Address correspondence to: Dr. Wai-Ping Fung-Leung, Johnson and Johnson Pharmaceutical Research and Development, 3210 Merryfield Row, San Diego, CA 92121. E-mail: wleung@prdus.jnj.com