Histamine H4 Receptor Mediates Chemotaxis and Calcium Mobilization of Mast Cells

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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 H1/H2 receptor antagonist thioperamide, but not by H1, or H2 receptor antagonists. This chemotactic response is mediated by the H4 receptor, because chemotaxis toward histamine was absent in mast cells derived from H4 receptor-deficient mice but was detected in H3 receptor-deficient mast cells. In addition, Northern blot analysis showed the expression of H4 but not H3 receptors on mast cells. Activation of H4 receptors by histamine resulted in calcium mobilization from intracellular calcium stores. Both Gq/11 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]-1H-pyrrole-2,5-dione (U73122). In summary, histamine was shown to mediate signaling and chemotaxis of mast cells via the H4 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 (H1, H2, and H3 receptor). Each receptor has its own expression pattern and mediates distinct effects: H1 receptors trigger smooth muscle contractions and are generally thought to play an important role in allergy. H2 receptors regulate gastric acid secretion in the stomach, and H3 receptors control the release of histamine and neurotransmitters by neurons. 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.

The amino acid sequence of the H4 receptor has low homology with other histamine receptors. Its closest member in the histamine receptor family is the H2 receptor that shares only a 35% amino acid homology with the H4 receptor, although the homology in the transmembrane region is 58%. However, the H4 receptor expression pattern is distinct from the H2 receptor. Although the expression of the H4 receptor is mainly restricted to cells in the central nervous system, the H4 receptor seems to be limited to cells of the hemopoietic lineage. The expression of the H4 receptor is mainly restricted to cells in the central nervous system (Lovenberg et al., 2000), the H4 receptor seems to be limited to cells of the hemopoietic lineage (Oda et al., 2000). The H4 receptor seems to be limited to cells of the hemopoietic lineage (Oda et al., 2000). The H4 receptor shows the expression of H4 but not H3 receptors on mast cells. Activation of H4 receptors by histamine resulted in calcium mobilization from intracellular calcium stores. Both Gq/11 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]-1H-pyrrole-2,5-dione (U73122). In summary, histamine was shown to mediate signaling and chemotaxis of mast cells via the H4 receptor. This mechanism might be responsible for mast cell accumulation in allergic tissues.

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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 H1/H2 receptor antagonist thioperamide, but not by H1, or H2 receptor antagonists. This chemotactic response is mediated by the H4 receptor, because chemotaxis toward histamine was absent in mast cells derived from H4 receptor-deficient mice but was detected in H3 receptor-deficient mast cells. In addition, Northern blot analysis showed the expression of H4 but not H3 receptors on mast cells. Activation of H4 receptors by histamine resulted in calcium mobilization from intracellular calcium stores. Both Gq/11 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]-1H-pyrrole-2,5-dione (U73122). In summary, histamine was shown to mediate signaling and chemotaxis of mast cells via the H4 receptor. This mechanism might be responsible for mast cell accumulation in allergic tissues.

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The amino acid sequence of the H4 receptor has low homology with other histamine receptors. Its closest member in the histamine receptor family is the H2 receptor that shares only a 35% amino acid homology with the H4 receptor, although the homology in the transmembrane region is 58%. However, the H4 receptor expression pattern is distinct from the H2 receptor. Although the expression of the H4 receptor is mainly restricted to cells in the central nervous system (Lovenberg et al., 2000), the H4 receptor seems to be limited to cells of the hemopoietic lineage (Oda et al., 2000). The H4 receptor seems to be limited to cells of the hemopoietic lineage (Oda et al., 2000). The H4 receptor shows the expression of H4 but not H3 receptors on mast cells. Activation of H4 receptors by histamine resulted in calcium mobilization from intracellular calcium stores. Both Gq/11 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]-1H-pyrrole-2,5-dione (U73122). In summary, histamine was shown to mediate signaling and chemotaxis of mast cells via the H4 receptor. This mechanism might be responsible for mast cell accumulation in allergic tissues.
et al., 2001). It was shown that specific H1 and H2 receptor antagonists and agonists do not bind to the H4 receptor. However, more typical H3 receptor ligands (such as thiomethyl amide, clobenproprit, imetit, and R-α-methylhistamine) could bind the H4 receptor with affinities different from that of the H3 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 H3 receptors mediate this action through Gαq proteins, resulting in calcium mobilization, H2 receptors signal through Gαs proteins, and cAMP increase, whereas H4 receptors signal through Gα0 proteins and inhibition of cAMP (Lovenberg et al., 1999). In the literature, two signaling pathways are thought to be used by the H4 receptor. First, using cells transfected with the H4 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 H3 receptor. Second, one study showed that histamine could not alter CAMP levels in H4 receptor-transfected cells, but instead increased calcium mobilization if the cells were cotransfected with Gαq/i1/2, Gαq/i3, or Gαx16 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 H4 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 prostanoids 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 H4 receptor on various purified hematopoietic cells and in various tissues was investigated. We showed that the mouse H4 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 H4 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 (Vancover, BC, Canada). RNasey 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). LTB4 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 H4 Receptor Gene Knockout Mice (H4, R−/−).** H4R−/− mice were generated by Lexicon Genetics (Woodlands, TX). A 9-kb mouse genomic fragment containing the mouse H4 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 H4 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 H4 receptor gene with oligonucleotides 5’-GAG ATG TAG ATG TGG TCG TTG G and 5’-CAT GTG CAG GCA CAC ACA TAC. The Southern blot of ES cell DNA digested with Ncol 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 H4 receptor gene were identified by PCR. Wild-type and H4 R−/− mice were obtained from cross-breeding of heterozygous mice.

**Detection of Mouse H4 Receptor Expression.** RNA from tissues and purified cells was prepared using a RNeasy kit according to the manufacturer’s instructions. H4 receptor RNA was detected by RT-PCR using specific mouse H4 receptor primers (5’-ATG TCG GAG TCT AAC AAT GCT GG and 5’-AGA AGA TAC TGA CTG GTT CGT 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 UV light. The amplified mouse H4 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 × 106/ml) were cultured at 37°C with 5% CO2 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
Daudi 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 H4 receptor cDNA clone (Liu et al., 2001a) and the purified RT-PCR product of the mouse H3 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 H4 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 RNasea kit, and 250 ng of RNA was used for the RT reaction according to manufacturer’s instructions. PCR using human H4 receptor-specific primers (5’-ACT AGA ATT CGC CAC CAT GCC AGC TAA TAG CAC and 5’-ATG GAT GAT CCA GCA TGG 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 H4 receptor gene knockout (H4R−/−), H3 receptor gene knockout (H3R−/−) 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 × 10⁷/ml) were cultured at 37°C with 5% CO₂ 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 1-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.
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<sub>3</sub>R<sup>-/-</sup>, H<sub>4</sub>R<sup>+</sup>-, and wild-type mice.

**Degranulation Assay.** Mast cells (5 × 10<sup>5</sup>/ml) were sensitized overnight with 2 μg/ml anti-DNP IgE. Mast cells (2 × 10<sup>5</sup>/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 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, cells received a second addition of 10 μM histamine. For PTX treatment, mast cells (1 × 10<sup>6</sup>/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<sup>5</sup>/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

CAMP Measurements. Mast cells (1 × 10<sup>5</sup>/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 agonist (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<sub>4</sub> Receptors.** Previously, we reported that the human H<sub>4</sub> receptor is expressed mainly in bone marrow and eosinophils (Liu et al., 2001a). In the present study, mouse H<sub>4</sub> expression was determined in different tissues and purified cell types of the hemopoietic lineage. Abundant expression of mouse H<sub>4</sub> receptor was detected in untreated mast cells and antigen-activated IgE-primed mast cells by RT-PCR (Fig. 2A). In contrast, H<sub>4</sub> 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<sup>+</sup> effector Th1 and Th2 cells, CD8<sup>+</sup> effector Tc1 and Tc2 cells, resting and LPS-activated B cells, as well as macrophages.

Expression of H<sub>4</sub> receptor in mast cells and eosinophils was further confirmed by Northern blot analysis (Fig. 2B and C). Both H<sub>1</sub> and H<sub>2</sub> receptors were detected on mast cells (data not shown), whereas H<sub>3</sub> receptor was undetectable by North-
ern blot analysis (Fig. 2C, left) and RT-PCR (data not shown). Consistent with the expression profile in mice, H4 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 H4 receptor is expressed on mast cells, basophils, and eosinophils.

H4 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 H1/H4 antagonist thioperamide and H1 antagonist diphenhydramine (data not shown), thereby excluding a role for H1, H3, or H4 receptors. However, the H2 receptor antagonist ranitidine could inhibit the histamine-induced cAMP increase. The results indicate that the histamine-induced cAMP increase in mast cells is H2 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 ED50 value of histamine-induced calcium mobilization was 3.8 μM. Neither H1 receptor antagonists nor H2 receptor antagonists altered the histamine-induced calcium mobilization.
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 \(\mu M\). This IC\(_{50}\) value is consistent with the relative binding affinities of histamine and thioperamide (Liu et al., 2001a). Together, the data suggest that H3 and/or H4 receptors are involved in calcium mobilization in mast cells.

Because mast cells do not express H3 receptors (Fig. 2C), it is likely that the calcium response is mediated by H4 receptors. A direct proof of H4 receptor-mediated calcium mobilization was demonstrated in mast cells from H4R\(^{-/-}\) and H3R\(^{-/-}\) mice. In contrast to wild-type mast cells, up to 30 \(\mu M\) histamine stimulation in H4R\(^{-/-}\) mast cells did not result in calcium mobilization (Fig. 4C). Mast cells from H4R\(^{-/-}\) mice showed an intermediate calcium response compared with mast cells from wild-type mice. This response is histamine-specific because H4R\(^{-/-}\) mast cells mediated normal calcium responses to ATP or ionomycin (data not shown). Furthermore, H4R\(^{-/-}\) 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 H4 receptor.

H4 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.

H4 Receptor Mediates Calcium Mobilization through Goi/o Proteins and PLC. To determine the G proteins used by H4 receptor in mast cells, Goi/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 Goi/o proteins are acting downstream of the H4 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 \(\mu M\), whereas the inactive analog U73343 (up to 10 \(\mu M\)) 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 H4 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 H4R\(^{-/-}\) mice was also unaffected by histo-
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. Thiopera-

mine pretreatment (Fig. 6B). In addition, thiopera-

mine 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 leukotri-

enes were also investigated. Compound 48/80 induced LT\( B_4 \) and prostaglandin release by mast cells, whereas histamine did not alter LT\( B_4 \) or prostaglandin levels (Table 1). In sum-

mary, \( H_4 \) 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 LT\( B_4 \) 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. Thiopera-

Table 1

| \( T \) (min) | Maximum | Untreated | Histamine | Histamine + Thiopera-

mine | Compound 48/80 |
<table>
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<tbody>
<tr>
<td>Prostaglandin</td>
<td>30</td>
<td>810 ± 16</td>
<td>28 ± 3</td>
<td>33 ± 0.3</td>
<td>34 ± 3</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>789 ± 98</td>
<td>50 ± 13</td>
<td>44 ± 5</td>
<td>37 ± 0.3</td>
</tr>
<tr>
<td>( LTB_4 )</td>
<td>30</td>
<td>56 ± 2</td>
<td>5 ± 0.7</td>
<td>6 ± 1.6</td>
<td>5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>58 ± 4</td>
<td>10 ± 3</td>
<td>8 ± 2</td>
<td>7 ± 0.5</td>
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In the present study, we showed that the mouse \( H_4 \) receptor is expressed on mast cells and eosinophils but not on other haemopoietic 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 re-

ported 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 thiopera-

mine as a specific \( H_3 \) antagonist (Kohn et al., 1994; Bissonnette, 1996), but recent data indicate that both the mouse and human \( H_4 \) receptor can bind thiopera-

mine and PL\( C \), 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 haemopoietic 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 thiopera-

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mine and PL\( C \), similar to that of the calcium response.
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, H4R−/− mast cells did not show any defects in degranulation, proliferation, or survival, indicating that the H4 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 H4 receptor. Antagonists of the H4 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 H4R−/− and H2R−/− mice, we demonstrated that histamine induced calcium mobilization from intracellular stores in mast cells via the H4 receptor. Calcium mobilization via the H4 receptor has also been observed using cells cotransfected with both the H4 receptor and chimeric G proteins (Morse et al., 2001). However, other studies using human H4 receptor-transfected cells have shown that hista-
inhibitor U73122, inhibited chemotaxis and calcium mobilization. Another IP3 receptor in the endoplasmic reticulum, which activates in mast cells through G protein, is known to occur in mast cells (Pacher et al., 2000).

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 Gai/o proteins. Possibly G protein βγ subunits dissociated from Gai/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.

Fig. 8. Signaling pathway of H_4 receptor on mast cells. Histamine binds to the receptor (1), which will activate Gai/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).

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References


Rhee (2001). It is therefore possible 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 Gai/o proteins and PLC are involved because PTX, which inactivates Gai/o proteins, and the PLC inhibitor U73122, inhibited chemotaxis and calcium mobilization. Gai/o proteins do not activate PLCβ, but G protein βγ subunits can activate PLCβ (Exton, 1996; Clapham and Neer, 1997; Rhee, 2001). It is therefore possible that PLCβ is activated by the G protein βγ subunits that are dissociated from Gai/o proteins when histamine binds to the H_4 receptor. Other G protein-coupled receptors have also been shown to signal via PLC and Gai/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 Gai/o proteins, phosphatidylinositol 3-kinase, Src, and Syk (Shefer and Sagi-Eisenberg, 2001). Stem cell factor is also known to induce calcium mobilization in mast cells involving activation of Gai/o proteins, phosphatidylinositol 3-kinase, p88 mitogen-activated protein, and mitogen-activated protein kinase kinases (Dastych et al., 1998; Sundstrom et al., 2001).

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