

Histamine H₄ receptor antagonists: The new antihistamines?

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Antihistamines (histamine H₁ receptor antagonists) are a mainstay treatment for atopic allergy, yet they are only partially effective in relieving the symptoms of the disease. They also have very limited value for the treatment of asthma, despite the well-characterized bronchoconstrictory effects of histamine. The recent discovery of a fourth histamine receptor (H₄), and the realization that it is exclusively expressed on hematopoietic cell types that are most implicated in the development and symptomatology of allergy and asthma, suggests that pharmacological targeting of the H₄ receptor, either alone or in combination with H₁ receptor antagonists, may prove useful for treating both allergy and asthma. Here we review the known biology associated with the H₄ receptor, as well the effects of a highly selective H₄ receptor antagonist.

Keywords Allergy, chemotaxis, eosinophils, histamine, histamine H₄ receptor, mast cells

Introduction

Histamine was first identified in 1910 as a mediator capable of constricting the guinea pig ileum [1]. It is widely expressed in many species, ranging from bacteria to mammals, where it exerts pleiotropic effects in a variety of physiological and pathological processes. Studies of the diverse biological effects of histamine have been greatly facilitated by the use of specific pharmaceutical agonists and antagonists, as well as gene-targeted animal models.

Some of the major physiological consequences of histamine, such as bronchoconstriction and vasodilation, are mediated by H₁ receptors and can be blocked by H₁ antihistamines [2,3]. In mice lacking the H₁ receptor, decreased allergic symptoms in rhinitis models and reduced eosinophil infiltration in conjunctivitis models have been reported [4,5]. Histamine also modulates immune responses by enhancing antigen-induced proliferation and differentiation of lymphocytes. The modulating effect is related to the H₁ and H₂ receptors, and is perturbed in mice that are deficient in either the H₁ or H₂ receptor [6,7].

In the gastrointestinal system, histamine is essential for gastric acid secretion and its effect is mediated by the H₂ receptor [8]. Gastric acid release in response to gastrin is impaired in mice deficient in either H₂ receptors or histidine decarboxylase (HDC), the enzyme that catalyzes histamine synthesis [9-11]. The H₂ receptor also influences other organ systems, including the vascular system where it exerts effects on vasodilation and tachycardia.

Histamine is a neurotransmitter of the central nervous system (CNS) and plays a role in learning and memory. The lack of histamine in HDC-deficient mice results in impaired memory, as well as defects in behavioral and sleep-wake controls [12-14]. Histamine effects in the brain are mediated by both H₁ and H₃ receptors, and the sedating effects of the first generation of H₁ antihistamines are caused by their penetration through the blood-brain barrier [15]. A reduction in locomotor activity and exploratory behavior are also observed in H₁ and H₃ receptor-deficient mice [16,17]. In addition, histamine acts as an anorexic factor that suppresses appetite and fat accumulation [18]. Regulation of energy homeostasis is abnormal in HDC-deficient mice, which are characterized by adiposity, impaired glucose tolerance and hyperleptinemia [19]. H₃ receptor-deficient mice manifest mild obese phenotypes, with reductions in energy expenditure and increases in body weight, food intake and adiposity [20]. Leptin-regulated feeding behavior is also significantly disturbed in H₁ receptor-deficient mice [18,21].

The recently identified H₄ receptor is expressed specifically in cells of the hematopoietic lineage, particularly in mast cells and eosinophils. Specific agonists and antagonists have been developed for the H₄ receptor [22••,23••,24••], and H₄ receptor gene-targeted mice have been generated [25••]. The biological roles of the H₄ receptor and the possible therapeutic use of H₄ receptor antagonists will be discussed.

Histamine synthesis and metabolism

Histamine is a biogenic amine converted from L-histidine by the cytosolic enzyme HDC, and is absent in HDC-deficient mice [26]. Histamine synthesized *de novo* is accumulated in secretory vesicles by the vesicular monoamine transporter subtype-2 (VMAT2). Defective storage and release of histamine can be detected in mast cells from VMAT2-deficient mice [27]. In peripheral tissues, mast cells and basophils are the most well-characterized histamine-producing cell types. In these cells, histamine is sequestered by sulfated heparin matrices and stored in granules as a preformed mediator that can be rapidly released upon cellular activation [28]. Some studies of HDC expression reveal a much broader distribution of histamine in other cell types, including macrophages, dendritic cells, T-cells, platelets, melanoma cells and embryonic stem cells [29-37]. Histamine is also produced in the brain, where binding of H₃ receptors leads to an autoregulatory feedback loop to control histamine release from pre-synaptic neurons [38]. In addition, histamine is secreted from the enterochromaffin-like cells in the gastric mucosa in response to gastrin [39].

Extracellular histamine levels can vary considerably and can reach high levels during generalized allergic reactions. Levels are controlled by degranulation from mast cells and basophils, as well as by *de novo* synthesis from other cell types. Metabolism of histamine, by the enzymes diamine oxidase (DAO) and histamine N-methyltransferase (HNMT) in particular, is also important for controlling the levels of circulating histamine [40,41]. While DAO is known to be a

Table 1. Comparison of the properties of histamine receptors.

Histamine receptor	H ₁	H ₂	H ₃	H ₄
Protein homology (%)	17	16	35	100
G α -protein association	G α_q	G α_s	G $\alpha_{i/o}$	G $\alpha_{i/o}$
Signaling	$\uparrow$ Ca ²⁺	$\uparrow$ cAMP	$\downarrow$ cAMP	$\uparrow$ Ca ²⁺
Expression	Ubiquitous	Ubiquitous	Neural cells	Mast cells, eosinophils, dendritic cells and T-cells
Key functions	Bronchoconstriction and vasodilation	Gastric acid secretion	Neurotransmitter release	Mast cell and eosinophil chemotaxis

secretory protein, HNMT appears to be a cytosolic enzyme that is devoid of a signal peptide sequence. In the CNS, histamine is metabolized primarily by HNMT, and it has been suggested that HNMT is associated with neuronal synaptosomes, from which it can readily access extracellular histamine for methylation [42]. Another possible mechanism of histamine clearance is through cellular uptake by specific transporters. Histamine uptake has been shown in macrophages [43], and an increase in histamine content in tissues of HDC-deficient mice is observed when these mice are maintained on histamine-enriched diets [44]. Certain transporters for neurotransmitters, such as the organic cation transporter-2 and the extraneuronal monoamine transporter, can also transport histamine into cells [45]. However, transporters specific for histamine have yet to be identified.

Expression of H₄ receptors

The diverse biological effects of histamine are mediated through different histamine receptors. Four different histamine receptors, namely H₁, H₂, H₃ and H₄ receptors, have been identified [38,46,47,48,49,50,51,52]. The different receptors have surprisingly low sequence homology, ranging from 16 to 35% at the protein level (Table 1).

H₁ and H₂ receptors are expressed on a large number of cell types and tissues, including vascular and bronchial smooth muscle cells, nerve cells, endothelial cells and several types of hematopoietic cells. H₃ and H₄ receptors have been postulated pharmacologically for some time, but have only recently been characterized at the molecular level. H₃ receptors are predominantly confined to the neural system [53,54]. In contrast, the H₄ receptor appears to be expressed almost exclusively on hematopoietic cells, particularly mast cells, basophils, eosinophils, dendritic cells and T-cells [25,48,49,50,52,55,56].

H₄ receptor-specific ligands

The affinity with which histamine binds to different histamine receptors varies significantly, with K_i values ranging from 5 to 10 nM, for the H₃ and H₄ receptors, to 2 to 10 μ M for the H₁ and H₂ receptors (Table 2). Pharmacological properties of the H₄ receptor have been characterized by using H₄ receptor-transfected cells [48,49,50,52,55]. As illustrated in Table 2, the classic H₁ and H₂ receptor antagonists, such as diphenhydramine, ranitidine and cimetidine, do not bind to the H₄ receptor. In contrast, most of the imidazole-based H₃ receptor ligands show various degrees of cross-reactivity with the H₄ receptor. This is not

surprising, given the shared sequence homology between the H₃ and H₄ receptors. The H₃ receptor agonist R- α -methylhistamine is also an agonist at the H₄ receptor, although it has approximately a 100-fold lower affinity (Table 2). The discrepancy between the known potency of R- α -methylhistamine versus the H₃ receptor, and its relative activity in eosinophils, was the main clue that led Raible *et al* to propose the existence of a fourth histamine receptor in 1994 [57]. Imetit has only a 10-fold difference in affinity between the two receptors and is a full agonist for both H₃ and H₄ receptors (Table 2). Clobenpropit (Vyzkumny Ustav Pro Farm a Bio Statni Podnik) is an antagonist for the H₃ receptor, but an agonist for the H₄ receptor, and can induce cellular responses in eosinophils via the H₄ receptor [56]. Thioperamide has been used as a specific H₃ antagonist in many studies. It is now known to bind the H₄ receptor with an affinity comparable to that at which it binds H₃ receptors [49] (Table 2). Thus, early reports of histamine effects particularly those observed in hematopoietic cells [57,58,59] that can be blocked by thioperamide could actually be mediated by H₄ receptors. In contrast to imidazole-based H₃ receptor ligands, the H₄ receptor appears to have limited cross-reactivity with non-imidazole-based H₃ receptor ligands [49,60-62]. The different histamine receptor ligands and their properties are listed in Table 2. A comprehensive description of the pharmacology of H₄ receptors can be found in a recent review by Jablonowski *et al* [63].

Selective imidazole-based agonists for the H₄ receptor have been reported recently [22] (Table 2). The chemical structures of these agonists are based on analogs of the H₃ receptor agonist, imfuramine. These compounds bind the H₄ receptor with K_i values of approximately 100 nM, and have approximately a 20-fold selectivity versus the H₃ receptor. Antagonists with a high degree of specificity for the H₄ receptor have also been reported [23,24] (Table 2). The best-characterized selective antagonist of those reported is JNJ-7777120 (Johnson & Johnson Pharmaceutical Research & Development LLC), which is from a series of indolylpiperazines. This compound has a K_i value of 4.5 nM in binding to the human H₄ receptor, and a pA₂ of 8.1 in antagonizing the effects of H₄ receptors on cAMP levels in transfected cells. It is equipotent against the human, mouse and rat receptors, exhibits at least 1000-fold selectivity over the H₁, H₂ or H₃ receptor and has no cross-reactivity against 50 other receptors and enzymes [24]. This compound has an oral bioavailability of approximately 30% in rats and 100% in dogs, with a half-life of approximately 3 h in both species. As described below, JNJ-7777120 blocks many H₄

Table 2. Histamine receptor ligands and their histamine receptor affinities.

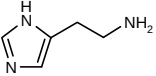
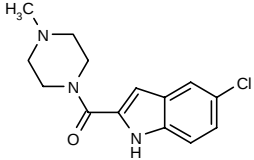
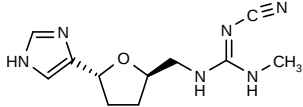
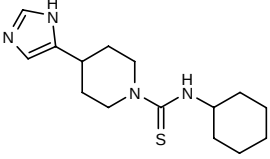
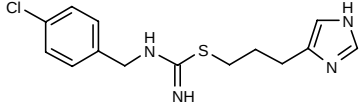
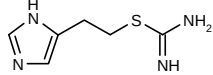
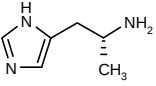
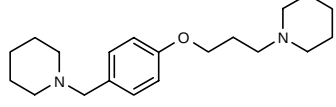
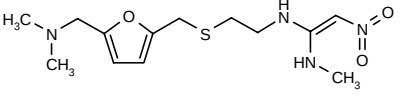
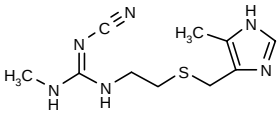
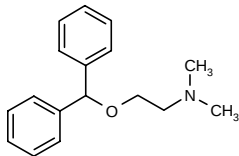
Compound	K _i (nM)				Mechanism of action	Reference
	H ₁	H ₂	H ₃	H ₄		
Histamine 	12,500	2000	5.4	8.1	Agonist	[49•,90,91]
JNJ-7777120 (Johnson & Johnson Pharmaceutical Research & Development LLC) 	> 10,000	> 30,000	5000	4.1	H ₄ antagonist	[24••]
OUP-16 (Univ Osaka Pharm Sci; Univ Osaka; Univ Vrije; Azwell Inc) 	-	-	2000	125	H ₄ agonist	[22••]
Thioperamide 	> 100,000	> 100,000	25	27	H ₃ /H ₄ antagonist	[49•,91,92]
Clobenpropit (Vyzkumny Ustav Pro Farm a Bio Statni Podnik) 	No affinity	No affinity	0.6	12.8	H ₃ antagonist, H ₄ agonist	[49•,93]
Imetit 	-	-	0.3	2.7	H ₃ /H ₄ agonist	[49•]
R-α-methyl-histamine 	-	-	0.7	146	H ₃ /H ₄ agonist	[49•]
JNJ-5207852 (Johnson & Johnson) 	> 10,000	> 10,000	0.57	> 10,000	H ₃ antagonist	[62•]
Ranitidine 	> 100,000	85	> 10,000	> 10,000	H ₂ antagonist	[49•,91,94]

Table 2. Histamine receptor ligands and their histamine receptor affinities (*continued*).

Compound	K _i (nM)				Mechanism of action	Reference
Cimetidine 	630,000	660	> 10,000	> 10,000	H ₂ antagonist	[49•,91,94]
Diphenhydramine 	15	-	> 10,000	> 10,000	H ₁ antagonist	[49•]

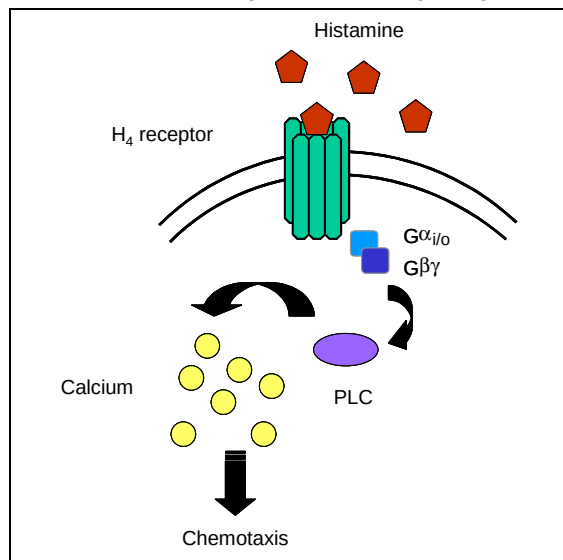
receptor-mediated functions, including histamine-induced chemotaxis in mast cells and eosinophils [24••,56••]. It also blocks histamine-induced migration of mast cells in mice, and significantly inhibits neutrophil infiltrations in three mouse peritonitis models induced by zymosan, urate crystal or the CXC chemokine, KC [24••]. These peritonitis models are all mast-cell-dependent, which suggests that the compound may be directly inhibiting mast cell function. The availability of selective ligands such as JNJ-7777120, provides a valuable tool for dissecting the physiological role of the H₄ receptor. In addition, these compounds may have a variety of therapeutic potentials.

Signaling mediated by H₄ receptors

Histamine receptors are G protein-coupled receptors with seven transmembrane (TM) domains. Two TM regions of the H₄ receptor, TM3 and TM5, are involved in histamine interaction, whereas another two, TM4 and TM6, play a role in receptor activation [64•]. As members of the G protein-coupled receptor family, all histamine receptors initiate signaling through interaction with specific G proteins. Activation of the H₁ receptor leads to changes in intracellular calcium levels via G_{α_q}-proteins, H₂ receptors signal through G_{α_s}-proteins leading to cAMP increases, and H₃ receptors activate G_{α_{i/o}}-proteins, resulting in inhibition of cAMP production [53,65] (Table 1). The signaling mechanisms for the H₄ receptor are still being explored. In transfected systems, an increase in intracellular calcium can be observed when the H₄ receptor is co-transfected with promiscuous or chimeric G proteins, such as G_{α_{q15}}, G_{α_{q11/2}}, G_{α_{q13}}, G_{α₁₅} and G_{α₁₆} [48•,49•,50•,52•]. In addition, activation of the H₄ receptor can inhibit forskolin-stimulated cAMP increases [48•,49•,52•,55•]. The effect on cAMP can be blocked by pretreating the cells with pertussis toxin (PTX), suggesting a role for G_{α_{i/o}}-proteins [48•] (Table 1). In transfected HEK-293 cells, the activation of the H₄ receptor can induce mitogen-activated protein kinase (MAPK) phosphorylation [50•]. This phosphorylation can be inhibited by PTX, implying that this effect is downstream of G protein activation. MAPK phosphorylation may also be downstream of calcium flux, since it is known that changes in intracellular calcium levels can activate this pathway [66]. The signaling mechanism appears to be similar for the mouse, rat and guinea pig H₄

receptors, where inhibition of cAMP has been shown in transfected SK-N-MC cells, as well as increased calcium mobilization when co-transfected with G_{α_{q15}} [67•].

In primary cells, H₄ receptor signaling is mediated by increases in intracellular calcium levels. Histamine induces calcium mobilization from intracellular stores, as well as an increase in cAMP in mouse primary mast cells [25••]. The calcium response is abolished in mast cells from H₄ receptor-deficient mice [25••], and can be blocked by the H₄ receptor-selective antagonist, JNJ-7777120, but not by antagonists for other histamine receptors [24••]. The cAMP response is completely attributed to H₂ receptor activation, since it is blocked by an H₂ receptor antagonist, but not by antagonists for other histamine receptors. This implies that in mouse mast cells, activation of the H₄ receptor leads to calcium mobilization and has no effect on cAMP levels. The calcium response in mast cells can be blocked by PTX and a phospholipase C inhibitor, U-73122 [25••]. These two inhibitors also block the H₄ receptor-mediated chemotaxis observed in these cells. The mobilization of calcium via phospholipase C activation is similar to that of the H₁ receptor [65]. However, while the H₄ receptor pathway is sensitive to PTX, the H₁ receptor pathway is not affected by it, suggesting the involvement of different mechanisms. This leads to a model for H₄ receptor-mediated signaling events (Figure 1). Histamine binding to the H₄ receptor recruits G_{α_{i/o}} proteins and releases Gβγ subunits from the G_{α_{i/o}}-protein complex. Phospholipase C is activated, probably by the released Gβγ subunits, and hydrolyzes phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol-1,4,5-trisphosphate (IP₃). IP₃ activates a calcium channel, possibly through an IP₃ receptor, to release calcium from intracellular stores, such as endoplasmic reticulum. In mouse mast cells, these events can culminate in the induction of chemotaxis [25••]. The same mechanism may also apply to eosinophils. Raible *et al* described histamine-mediated calcium mobilization in human eosinophils, and this is now known to be due to activation of the H₄ receptor [57•]. More recently, it has been shown that H₄ receptor activation leads to increases in intracellular calcium levels in eosinophils, and results in chemotaxis and adhesion molecule upregulation that are blocked by PTX [68••].

Figure 1. A model of H_4 receptor-mediated signaling events.

Histamine binding to the H_4 receptor recruits $G_{\alpha i/o}$ -proteins and releases $G_{\beta\gamma}$ -proteins. PLC is activated by $G_{\beta\gamma}$ -proteins and triggers the release of calcium from intracellular stores, which eventually leads to chemotaxis. (Reproduced with permission from the American Society for Pharmacology and Experimental Therapeutics and Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung W-P: **Histamine H_4 receptor mediates chemotaxis and calcium mobilization of mast cells.** *J Pharmacol Exp Ther* (2003) **305**(3):1212-1221. © 2003 ASPET.)

It is interesting to speculate on the possible interactions of the signaling pathways mediated by the different histamine receptors, since many cells express more than one type. This is especially true of hematopoietic cells, since eosinophils, dendritic cells and T-cells, like mast cells, may express H_1 , H_2 and H_4 receptors. Upon exposure to histamine, different histamine receptors could be activated simultaneously and interactions between the different pathways may be expected. For example, both the H_1 and H_4 receptors lead to increases in intracellular calcium levels, albeit through different pathways. Therefore, it may be expected that in some instances there could be synergy between the two receptors. This interaction may be controlled by the differences in the affinity for histamine among the receptors. The H_4 receptor has the highest affinity, followed by the H_2 and then the H_1 receptors (Table 1). Hence, it would be expected that the H_4 receptor could be activated at low histamine levels initially, followed by the H_2 and then the H_1 receptors as histamine levels increase. The other mechanism of regulation may be the desensitization of histamine receptors. Exposure to histamine or pharmaceutical agonists can cause desensitization of histamine receptors via different mechanisms, including receptor internalization, changes in signaling events or gene induction [51•,56••,69,70]. Thus, different histamine receptors could be activated over different ranges of histamine concentrations. This can be seen in the early research of Clark *et al*, who showed that histamine could induce eosinophil chemotaxis over a narrow concentration range, but was inhibitory at higher concentrations [71•,72]. The inhibition was mediated by activation of H_2 receptors, and more recent studies suggest that the stimulatory effect is due to activation of H_4 receptors [56••,68••,73•].

Role of H_4 receptors on mast cells

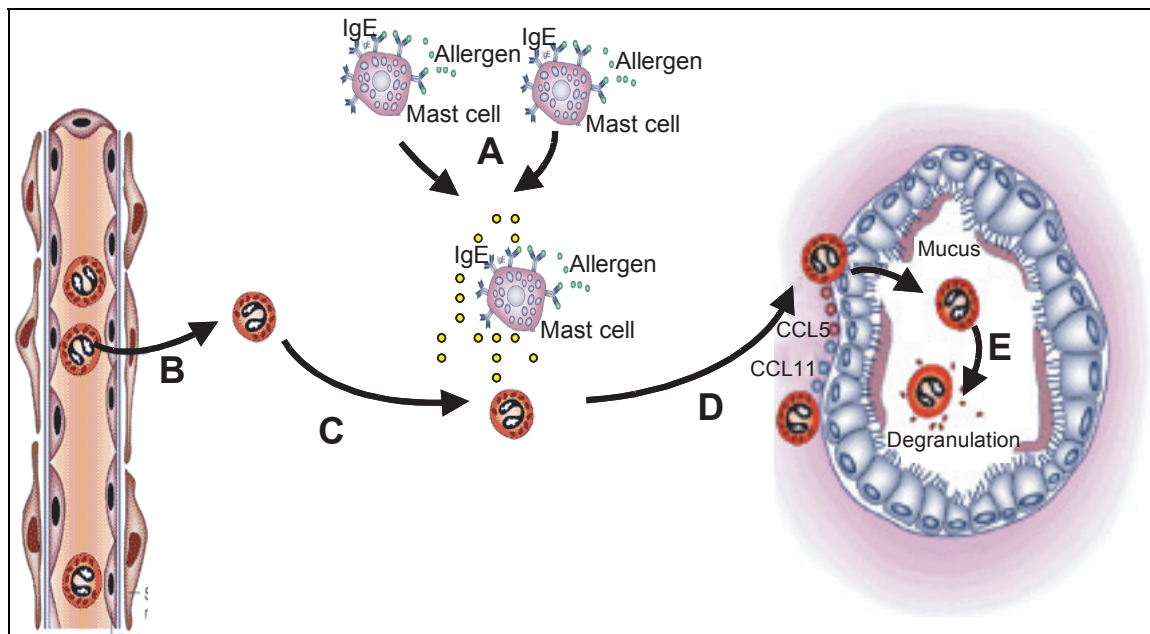
Mast cells are the key effector cells in allergic diseases. Mast cells bind immunoglobulin (Ig)E through IgE receptors, and their subsequent exposure to antigens triggers IgE receptor cross-linking that leads to degranulation. Histamine is one of the major mediators produced by mast cells, yet these cells also express H_4 receptors [25••], suggesting a possible effect of histamine on mast cells themselves.

Histamine appears to be important for mast cell development, and possibly also for mast cell functions. HDC-deficient mice are completely devoid of histamine, and these mice have fewer numbers of mast cells in their tissues. In addition, these cells have reduced granular contents and are defective in degranulation [26,74,75]. In contrast, normal distribution of mast cells is found in H_4 receptor-deficient mice, suggesting that the effect of histamine on mast cell development is not through the H_4 receptor [C Hofstra, unpublished data].

Within the histamine receptor family, the H_3 receptor has the highest homology to the H_4 receptor in protein sequence, and they both bind histamine with high affinities (Tables 1 and 2). The H_3 receptor is known to be an autoreceptor on neural cells [38,76], and H_4 receptors may have similar feedback roles in the storage and secretion of histamine in mast cells. Studies of H_4 receptor-deficient mice, however, reveal that mast cells from these mice have normal histamine content, and that they respond to antigen-IgE complex stimulation with proper degranulation and histamine release, ruling out the feedback regulatory role of the H_4 receptor on mast cells [25••]. Furthermore, addition of histamine to mast cells does not trigger mast cell degranulation, nor does it modulate mast cell degranulation triggered by the antigen-IgE complex [25••]. Consistent with the *in vitro* findings in mast cells, normal passive anaphylactic shock has also been observed *in vivo* in H_4 receptor-deficient mice. These mice show a normal 'wheal and flare' erythema response on the skin, after intradermal injection of IgE and antigens for local mast cell degranulation [C Hofstra, unpublished data].

In an allergic reaction, large amounts of histamine are released locally from mast cells at sites of allergen exposure. Accumulation of mast cells and basophils at sites of allergic reactions has been reported in patients with chronic allergic diseases, and the extent of accumulation often correlates with disease severity [77,78]. It is possible that one of the functions of histamine is to recruit mast cells and basophils to sites of allergic reactions. Histamine induces mast cell chemotaxis via the H_4 receptor, and the effect is completely abolished in mast cells from H_4 receptor-deficient mice [25••]. This chemotaxis can also be blocked by thioperamide and by the H_4 receptor-specific antagonist JNJ-777120, but not by antagonists for other histamine receptors [24••,25••]. *In vivo* migration of mast cells toward histamine can be seen in mice after histamine inhalation. Mast cells in the trachea of these mice migrate from connective tissues toward the epithelium, but this can be blocked significantly by JNJ-777120 [24••]. It appears that histamine is a chemotactic factor that recruits mast cells to sites of allergic reactions. The same effect may also apply to basophils, which are

Figure 2. A model of histamine effects on eosinophil infiltrations.



(A) Histamine released by degranulated mast cells mediates mast cell accumulation in lung tissues. (B) Eosinophils are attracted by chemokines produced by blood vessel endothelial cells and exit from blood circulation. (C) Histamine in lung tissues mediates eosinophil chemotaxis to the site of mast cell degranulation. (D) Histamine-primed eosinophils are attracted by chemokines produced by bronchial epithelial cells and migrate to bronchial alveoli. (E) Eosinophil degranulation occurs in bronchial alveoli.

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known to express H₄ receptors [25••]. The process of mast cell and basophil accumulation could be important for the amplification of allergic reactions that eventually leads to the development of allergic diseases (Figure 2). Interruption of this process with H₄ receptor antagonists may be a novel and effective treatment to attenuate the development of allergic diseases.

Both thioperamide and JNJ-777120 have reduced neutrophil infiltrations in mast cell-dependent peritonitis models [24••,79•]. The prevention of neutrophilia by H₄ receptor antagonists is probably due to their inhibitory effects on leukotriene B₄ production by mast cells [79•]. These findings suggest that in addition to chemotaxis, the H₄ receptor may mediate other biological effects on mast cells. The therapeutic effects of H₄ receptor antagonists may extend to general inflammations.

Role of H₄ receptors on eosinophils

Eosinophils are bone marrow-derived granulocytic leukocytes that normally reside in tissues, especially in the respiratory and intestinal systems and in the uterus. They can be activated by an extensive range of biological stimulants including chemokines, cytokines and lipid mediators, which induce a variety of cellular responses, such as cell adhesion, chemotaxis, respiratory burst, mediator secretion and degranulation [80,81]. Accumulation and inappropriate activation of eosinophils result in the release of toxic granule proteins, which are thought to cause airway epithelial damage and the development of bronchial hyper-reactivity in asthma [77,82,83].

In an allergic reaction, eosinophil infiltration occurs in the late phase, whereas mast cell degranulation and histamine release happen in the early phase. The possible role of histamine in the recruitment and function of eosinophils has been investigated. When eosinophils are treated with histamine, superoxide ion generation is induced by H₁ receptors, whereas degranulation is downregulated by H₂ receptors [84,85]. Early studies showed an effect of histamine on eosinophil chemotaxis via non-H₁ and non-H₂ receptors [71•,72,86]. This chemotactic response is now known to be mediated by the H₄ receptor, which is expressed at high levels in eosinophils [25••,49•,56••]. The response can be blocked by thioperamide and JNJ-777120 [56••,73•].

In comparison to other typical eosinophil chemokines, histamine is not a highly potent chemotactic factor for eosinophils [56••]. Histamine concentrations in blood circulation may not be sufficient to drive eosinophil migration. However, histamine in tissues, particularly in areas with local mast cell degranulation, may reach the critical concentrations for eosinophil chemotaxis. Histamine also enhances chemotactic responses of eosinophils to other chemokines such as eotaxin [56••,68••]. Such an adjuvant effect of histamine could be important for priming eosinophils to respond to other chemokines. This leads to a model for the chemotactic effects of histamine on eosinophils, as illustrated in Figure 2. Migration of eosinophils to sites of allergic reaction, such as the respiratory system, may be through a series of sequential chemotactic events mediated by different stimuli. Chemokines produced by blood vessel endothelial cells are responsible for diapedesis of eosinophils from blood circulation into tissues. In tissues,

histamine released by degranulating mast cells not only recruits other mast cells and basophils, but also attracts eosinophils to sites of allergic reactions. Eosinophils accumulated in tissues are then primed by histamine and respond to other chemokines produced by the bronchial epithelium, such as eotaxin. Attracted by chemokines from the bronchial epithelium, eosinophils migrate into bronchial alveoli, where they degranulate and cause damage to the respiratory system.

Chemotaxis is a directional cell movement towards a chemoattractant gradient, which requires an establishment of cell polarity, and thus a cell shape change towards a directional signal. Polymerization of G-actin to F-actin is an intracellular event that leads to cell shape change and locomotion. Actin polymerization is induced in histamine-treated eosinophils and this effect can be blocked by thioperamide, suggesting that it is mediated by the H_4 receptor [68••]. The analysis of eosinophil cell-shape change with flow cytometry, known as a gated autofluorescence forward scatter (GAFS) assay, allows a quantitative measurement of cell shape change induced by chemoattractants [87]. Using the GAFS assay, histamine can induce a rapid shape change in eosinophils via the H_4 receptor. This can be blocked by thioperamide and JNJ-777120 [56••,68••]. Similar eosinophil shape change can be induced by imetit and clobenpropit, which are known H_4 receptor agonists [56••,68••]. The change can also be detected in whole blood samples without the need for eosinophil purification, and therefore could be a useful biomarker for clinical trials of H_4 receptor antagonists. The eosinophil shape change induced by histamine is rapid but transient. A shape change occurs after a 1-min incubation with histamine and a maximal response is reached at 5 min. Removal of histamine abolishes the eosinophil shape change immediately. In addition, eosinophils can be desensitized by histamine and these cells become refractory to further histamine re-stimulation. The mechanism of H_4 receptor desensitization is still unclear. It may involve different mechanisms including receptor internalization, activation of intracellular signaling events or gene inductions, which have been reported for other histamine receptors [51•,69,70].

In addition to actin polymerization and establishment of cell polarity, cell-cell interactions via adhesion molecules play an important role in cell migration. The phases of leukocyte migration are comprised of adhesion, spreading, diapedesis through the vessel endothelial cells, and infiltration into tissues [88]. Upregulation of adhesion molecules on the cell surface is essential for cell spreading and diapedesis. Histamine increases cell surface expression of the adhesion molecules CD11b/CD18 (Mac-1) and CD54 (intercellular adhesion molecule-1) on eosinophils [56••,68••]. This effect is mediated by the H_4 receptor and can be blocked by thioperamide and the H_4 receptor-specific antagonist JNJ-777120 [56••,68••]. In summary, histamine acting on the H_4 receptor induces eosinophil chemotaxis, which is accompanied by cell-shape change, actin polymerization and adhesion molecule upregulation.

Conclusions: Therapeutic implications of H_4 receptor antagonists

The role of histamine in atopic allergy is well known and H_1

receptor antagonists are widely used to treat this pathological condition. However, their effects are moderate and reduce allergic symptoms only by 40 to 50%. In particular, H_1 receptor antagonists do not relieve nasal congestion. In contrast, topical steroids effectively address all allergic symptoms, including nasal congestion. Despite this, steroids are not commonly used for treatment of allergies. This is because of their potential side effects, slow onset of action and general public resistance to their use. The H_4 receptor-mediated effects of histamine on mast cells and eosinophils, both of which are important effector cells in the allergic response, suggest that H_4 receptor antagonists could relieve some of the acute allergic symptoms. The finding that H_4 receptor antagonists inhibit histamine-induced migration of mast cells and eosinophils, both *in vitro* and *in vivo*, raises the possibility that this class of compounds could reduce the allergic hyper-responsiveness developed upon repeated exposure to antigens. This hyper-responsiveness is characterized by increased sensitivity to allergens, as well as an increase in the number of mast cells and other inflammatory cells in the nasal mucosa.

Histamine clearly has the ability to induce bronchoconstriction in asthmatic patients, and many studies have suggested that histamine is indeed involved in the pathogenesis of asthma. In support of this hypothesis, mast cell and basophil numbers are increased in bronchial epithelium and bronchioalveolar lavage from asthmatic patients. However, H_1 receptor antagonists have demonstrated only limited efficacy in the treatment of asthma, thus the interest in histamine in relation to asthma has decreased in recent years. The possibility that H_4 receptors, in addition to H_1 receptors, may be important for the histamine-mediated aspects of the disease progression and symptoms, may shift the attention back to histamine.

It is possible that H_4 receptors play a more general role in inflammation, since they can be involved in neutrophil infiltration *in vivo* [24••,79•]. In addition, a role for the H_4 receptor in interleukin-16 release from CD8+ T-cells has been reported [89•]. This finding, coupled with the fact that H_4 receptors are expressed on both dendritic cells and T-cells, opens up the possibility that H_4 antagonists (or agonists) may prove useful for the treatment of a variety of immune-mediated diseases. This, of course, warrants more study both in animal models and clinically.

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