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PII: S0014-2999(18)30491-6
DOI: <https://doi.org/10.1016/j.ejphar.2018.08.028>
Reference: EJP71953

To appear in: *European Journal of Pharmacology*

Received date: 2 May 2018
Revised date: 20 August 2018
Accepted date: 23 August 2018

Cite this article as: Linda J Kay, S Kim Suvarna and Peter T Peachell, Histamine H₄ receptor mediates chemotaxis of human lung mast cells, *European Journal of Pharmacology*, <https://doi.org/10.1016/j.ejphar.2018.08.028>

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Histamine H₄ receptor mediates chemotaxis of human lung mast cells

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Abstract

The diverse effects of histamine are mediated by discrete histamine receptors. The principal repository of histamine in the body is the mast cell. However, the effects of histamine on mast cells, especially those of human origin, have not been fully elucidated. In this study, the expression of histamine receptors in human lung mast cells was evaluated. Moreover, the effects of histamine receptor engagement on both mediator release and chemotaxis were investigated. Mast cells were isolated and purified from human lung tissue. Histamine receptor expression was determined by RT-PCR and q-PCR. Both methods for the detection of histamine receptors were in accordance and human lung mast cells expressed mRNA for histamine H₄ and

histamine H₁ receptors, variably expressed histamine H₂ receptor but did not express histamine H₃ receptor. The effects of selective histamine receptor agonists on the release of both pre-formed (histamine) and newly-synthesized (cysteinyl-leukotriene, prostaglandin D₂) mediators were investigated. None of the agonists tested had any direct effects on mediator release. None of the agonists modulated release stimulated by anti-IgE. Further studies showed that histamine induced migration of mast cells. Chemotaxis appeared to be mediated by the histamine H₄ receptor since JNJ28610244 (H₄ agonist) was chemotactic for mast cells whereas 2-(2-pyridyl) ethylamine (H₁ agonist) was not. Furthermore, the selective histamine H₄ receptor antagonist, JNJ7777120, effectively reversed the chemotaxis of mast cells induced by JNJ28610244. Overall, these experiments identify the histamine H₄ receptor as chemotactic for human lung mast cells. This mechanism might influence mast cell accumulation in the lung.

Keywords mast cells; histamine; histamine H₄ receptor; chemotaxis

1. Introduction

Histamine is known to regulate many pathophysiological processes. As examples, histamine has been shown variously to promote allergic inflammation, to induce acid secretion in the gut and to modulate neurotransmission in the central nervous system (Panula et al., 2015). These effects of histamine are mediated by the histamine H₁, histamine H₂ and histamine H₃ receptors, respectively. Historically, the histamine H₁ and histamine H₂ receptors have served as targets for therapeutically useful drugs, H₁ anti-histamines to treat allergic symptoms (Simons and Simons, 1994) and H₂ antagonists to counter excess acid secretion (Hershcovici and Fass, 2011). Targeting

the histamine H₃ receptor has led to the development of selective ligands involved in modifying behavioural disorders (Schwartz, 2011).

More recently, a fourth receptor, the histamine H₄ receptor, has been identified (Nakamura et al., 2000; Oda et al., 2000). Particular interest in the histamine H₄ receptor has developed since a body of emerging data suggests an intimate association of the receptor with diseases that have an allergic basis including atopic dermatitis and asthma (Thurmond et al., 2008) as well as certain auto-immune disorders such as arthritis and multiple sclerosis (Cowden et al., 2013; Liu, 2014; Thurmond, 2015; Kim et al., 2017). Although the histamine H₁ receptor has traditionally been associated with allergic reactions, the failure of H₁ anti-histamines to provide much symptomatic relief in conditions such as asthma (Simons, 1999) could suggest the participation of an additional or alternative receptor which may involve the histamine H₄ receptor (Liu, 2014). Supporting this contention, studies have demonstrated that the histamine H₄ receptor can activate a wide variety of pro-inflammatory cells (Thurmond et al., 2008). This has led to the development of histamine H₄ receptor antagonists as potential therapeutic agents for diseases with an allergic basis (Thurmond et al., 2008; Walter et al., 2011; Liu, 2014; Thurmond, 2015).

The mast cell has long been recognised as central to the mediation of allergic responses not least because it is the principal repository of histamine in the body. Following activation by allergens, the mast cell can release histamine as well as generate a host of additional mediators such as cysteinyl-leukotrienes (cys-LTs) and prostaglandin D₂ (PGD₂). These mediators act in concert to promote inflammation and, if mast cell activation occurs in the lung, these mediators can promote potent bronchoconstriction (Bingham and Austen, 2000; Bradding et al., 2006).

There have been a few studies investigating the effects of histamine on mast cells but the majority of these has been on rodent and cultured mast cells (Hofstra et al., 2003; Lippert et al., 2004; Jemima et al., 2014). How representative these systems are of primary human cells is a moot point since a great deal of heterogeneity is known to exist among mast cells isolated from different species and from different sites of the same species (Pearce, 1983; Lowman et al., 1988). In addition, an added layer of complexity exists since it is known that, despite similarities in amino acid sequence among orthologues of rodent and human histamine receptors, functional responses to given ligands can be markedly different (Leurs et al., 2009; Lim et al., 2010).

The aim of this study, therefore, was to investigate the potential regulation of human lung mast cells by histamine receptors with particular emphasis on the role that the histamine H₄ receptor might play.

2. Materials & Methods

2.1 Buffers

A supplemented phosphate buffered saline (PBS) was employed in this study. +PBS contained (mM): NaCl 137; Na₂HPO₄·7H₂O 8; KCl 2.7; KH₂PO₄ 1.5; CaCl₂·2H₂O 1; MgCl₂·6H₂O 1; glucose 5.6; and supplemented with human serum albumin (HSA) 30 µg/ml. The pH was titrated to 7.3.

2.2 Preparation of compounds

2-(2-pyridyl) ethylamine, amthamine dihydrobromide and methimepip dihydrobromide were prepared as stock solutions (10⁻¹ M) in water. JNJ28610244

was prepared every 2 months and JNJ7777120 monthly as stock solutions (10^{-2} M) in dimethyl sulphoxide (DMSO). All stock solutions were stored at -20°C in small aliquots except 2-(2-pyridyl) ethylamine which was stored at room temperature. Histamine was prepared daily as an aqueous solution as a 10^{-2} M stock. Stem Cell Factor (SCF) was prepared as a stock solution (100 $\mu\text{g}/\text{ml}$) in water and stored frozen in small aliquots. Anti-human IgE antibody was stored frozen but a working aliquot was stored at 4°C for a month.

2.3 Lung tissue

Human lung tissue was obtained following surgical resections. Informed written consent was obtained from participants. The study was approved by the National Research Ethics Service (Sheffield Lung Research Tissue Bank [Version 2.1]; REC reference: 15/NW/0657).

2.4 Cell isolation

Mast cells were isolated from human lung tissue using methods described in detail elsewhere (Lewis et al., 2013). In brief, macroscopically normal tissue from lung resections was disrupted physically and enzymatically to generate a mixed cell suspension. Mast cells were visualized by microscopy using an alcian blue stain (Gilbert and Ornstein, 1975). Alcian blue has been used extensively to identify mast cells in a mixed cell population (for example, Schulman et al., 1983). The purity of mast cells was then enhanced by flotation over discontinuous Percoll gradients (Weston et al., 1997) resulting in purities ranging from 10 - 40 % (median, 28% purity). These preparations of mast cells of enhanced purity were used in functional studies and for chemotaxis. Mast cells were further purified using a MACS magnetic

cell sorting system (Miltenyi Biotec, Surrey, UK) according to the manufacturer's instructions. In brief, Percoll preparations of mast cells were incubated with magnetic microbeads tagged with monoclonal antibody directed at human CD117 (clone AC126). Cell separations were performed over an LS column. Full details of the procedure are provided elsewhere (Havard et al., 2011). Mast cells of high purity (90 - 98%) were generated by this method and these preparations were used in RT-PCR and q-PCR experiments.

2.5 Mediator release

Mediator release experiments were performed in +PBS buffer using methods that have been described previously (Lewis et al., 2013). Mast cells were incubated with or without histamine receptor agonist for 10 min before challenge with anti-IgE (0.2 µg/ml) for 25 min at 37 °C. Cells were then pelleted by centrifugation (480 x g, room temperature, 4 min). Histamine released into the supernatant was determined by an automated spectrofluorometric method (Ennis, 1991). Cells incubated in buffer alone served as a measure of spontaneous histamine release. Cells lysed in perchloric acid (1.6%) provided a measure of total histamine content. Histamine release was expressed as a percentage of the total histamine content. The generation of cysteinyl-leukotrienes (cys-LTs) and prostaglandin D₂ (PGD₂) was monitored using commercially available ELISA kits (Cayman Chemical Company, Ann Arbor, MI, USA). All experiments were performed in duplicate.

2.6 Chemotaxis

Chemotaxis assays were performed using the ChemoTx® system (96 well plate, 8 µm pore size). Assays were performed in RPMI-1640 buffer supplemented with bovine

serum albumin (BSA; 0.1%). To the bottom chamber, histamine, alternative receptor selective agonists or antagonist were added. To the top chamber, mast cells were added. Stem Cell Factor (SCF), a known mast cell chemoattractant, was also used in these experiments as a positive control. A chemokinesis control was also included in all experiments and in this situation, SCF, agonist or antagonist was present in both the top and bottom chambers. An additional control of media in the bottom chamber and mast cells in the top chamber served as a measure of the random movement of mast cells. After incubating the plate for 4 h at 37 °C in a CO₂ incubator, the number of mast cells that had migrated into the bottom well were visualized by microscopy using an alcian blue stain (Gilbert and Ornstein, 1975). In order to assess the reliability of determining mast cell chemotaxis by microscopy, confirmatory experiments were performed in which cells migrating in response to SCF were either counted by microscopy using the alcian blue stain or were lysed with perchloric acid (1.6%) and the histamine content determined by automated spectrofluorometry. There was good correspondence between the two methods in assessing mast cell migration (see Fig. S1; Supporting Information repository). All chemotaxis experiments were performed in duplicate.

2.7 RT-PCR

RNA was extracted from purified mast cells ($1 - 3 \times 10^6$ cells) by adding Tri-reagent (1 ml). In order to generate cDNA, samples were processed as described previously (Kay et al., 2013). The cDNA was then amplified using primers specific to regions of the human histamine receptor subtypes by PCR (Wang et al., 2000; Lippert et al., 2004). Expression of the housekeeping gene, β -actin, was also evaluated. Primers were synthesised by Sigma (Poole, UK). The primer pairs that were used can be

found in Table S1 (Supporting Information repository). Cycling conditions for the histamine receptor subtypes were 95 °C for 2 min, 95 °C for 45 s, see Table S1 for annealing conditions, 72 °C for 60 s, with a final elongation step of 72 °C for 10 min. For β -actin, cycling conditions were 94 °C for 2 min, 95 °C for 30 s, see Table S1 for annealing conditions, 72 °C for 35 s, with a final elongation step of 72 °C for 7 min. All PCR reactions consisted of 35 cycles. PCR products were visualised with ethidium bromide on 2 % agarose gels alongside Quick-Load 100 bp DNA ladder (New England Biolabs, Hitchin, UK). To ensure the correct amplification had taken place, PCR products were cut from the agarose gel, cleaned as previously described (Kay et al., 2013) before being subjected to in-house genotypic analysis by automated sequencing (ABI 3730 DNA Analyser; Applied Biosystems, Carlsbad, CA, USA).

2.8 Quantitative PCR

Quantitative PCR (q-PCR) was also carried out on the extracted cDNA using TaqMan gene expression assay probes (Applied Biosystems, Carlsbad, CA, USA) specific for the human histamine receptor subtypes alongside the housekeeping gene, β -actin. Details are provided in Table S2 (Supporting Information repository). Reaction mixtures consisted of 10 μ l 2x PCR Master Mix (Eurogentec, Southampton, UK), 1 μ l 20x probe set and 8 μ l nuclease free water. Nineteen microliters of this master mix was added to each well of the q-PCR plate alongside 1 μ l of cDNA template. Reactions were carried out using an ABI 7900 automated TaqMan (Applied Biosystems, Carlsbad, CA, USA) with conditions consisting of 50 °C for 2 min, 94 °C for 10 min, and 40 cycles of 95 °C for 15 s and 50 °C for 1 min.

2.9 Materials

The following were purchased from the sources indicated; 2-(2-pyridyl) ethylamine, amthamine, BSA, histamine, HSA, Percoll (Sigma, Poole, UK); JNJ7777120, methimepip (Tocris, Bristol, UK); RPMI-1640 (Invitrogen, Paisley, UK); anti-human IgE, clone HP6061 (Stratech Scientific Ltd, Newmarket, UK); anti-human CD117, clone AC126 (Miltenyi Biotec, Surrey, UK); SCF (Peprotech, Rocky Hill, NJ, USA). All other reagents were from Sigma (Poole, UK).

JNJ28610244 was a kind gift from Janssen Research & Development, LLC (San Diego, USA).

2.10 Data analysis

Data are expressed as means $\pm$ S.E.M. Statistical significance was assessed utilising repeated measures ANOVA. Post hoc analysis was performed using Dunnett's test.

3. Results

3.1 Histamine receptor expression

Expression of histamine receptors in human lung mast cells was determined by RT-PCR. There was evident expression of mRNA for the histamine H₄ and histamine H₁ receptors in both human lung mast cells and the human mast cell line, LAD2 (Fig. 1). Expression of histamine H₂ receptor was more variable among lung mast cell preparations but absent in LAD2 cells. Neither lung mast cells nor LAD2 cells expressed histamine H₃ receptor. However, in separate experiments, we could readily demonstrate the presence of histamine H₃ receptor in several breast cancer cell lines, MDA-MB-468, MDA-MB-231, ZR-75-1 and MCF7 (Medina et al., 2008; Tanaka et

al., 2016). These RT-PCR experiments were further supported by q-PCR experiments in which expression of histamine H₄ and histamine H₁ receptors and some histamine H₂ receptor was observed in human lung mast cells whereas the histamine H₃ receptor was not expressed (Fig. 2).

3.2 Functional responses

In further experiments, the effects of selective agonists at histamine receptors on mast cell responses were determined. The agonists studied were 2-(2-pyridyl) ethylamine (H₁ agonist), amthamine (H₂ agonist), methimepip (H₃ agonist) and JNJ28610244 (H₄ agonist). The effects of these agonists alone and in combination with a half-maximal releasing concentration of anti-IgE (0.2 µg/ml) were determined. None of the agonists tested had any effect alone or in combination with anti-IgE on the release of the pre-formed mediator histamine (Fig. 3A). Moreover, none of the agonists influenced the production of either cys-LTs (Fig. 3B) or PGD₂ (Fig. 3C), mediators that are generated *de novo*.

3.3 Chemotaxis

The effects of histamine on chemotaxis of human lung mast cells were explored. In preliminary experiments, SCF, which has previously been shown to induce chemotaxis in a variety of different mast cell types (Meininger et al., 1992; Nilsson et al., 1994; Reber et al., 2006; Draber et al., 2016), was studied. SCF was consistently effective at inducing human lung mast cell chemotaxis (Fig. 4A). Alongside SCF, the effects of histamine on chemotaxis of mast cells were investigated. Histamine was found to be chemotactic for mast cells (Fig. 4B).

Since histamine is likely to engage all subtypes of histamine receptor if expressed by mast cells, further studies were performed to evaluate whether this effect of histamine might be mediated by a particular histamine receptor. The effects of the histamine H₁ receptor agonist, 2-(2-pyridyl) ethylamine, and that of the histamine H₄ receptor agonist, JNJ28610244, were investigated. The data show that JNJ28610244 was chemotactic for mast cells whereas 2-(2-pyridyl) ethylamine was not (Fig. 5).

To investigate further the specificity of the effect observed with JNJ28610244, the influence of the histamine H₄ receptor antagonist, JNJ7777120, on chemotaxis was evaluated. The data show that JNJ7777120 effectively reversed the chemotaxis of mast cells induced by the histamine H₄ receptor agonist, JNJ28610244 (Fig. 6).

4. Discussion

In the present study, we have assessed whether histamine receptors modulate the function of human lung mast cells. The principal finding of this paper is that the histamine H₄ receptor mediates migration of human lung mast cells.

Initial experiments were performed to determine the expression of histamine receptor subtypes in human lung mast cells. Our studies, using both RT-PCR and q-PCR, demonstrated that lung mast cells appear to express histamine H₄ and histamine H₁ receptors prominently, histamine H₂ receptors more variably among preparations and are devoid of histamine H₃ receptors. These statements of the relative expression

levels of histamine receptor genes should be treated with caution since it is known that the efficiency of PCR amplification can be influenced by the primer pairs selected. Nonetheless, it is noteworthy that disparate methods, RT-PCR and q-PCR, showed similarity in the expression pattern of histamine receptors.

A wide variety of mast cells isolated from different species, cultured mast cell lines and human basophils all appear to express histamine H₄ receptors with variable expression of histamine H₁ and/or histamine H₂ receptors reported (Hofstra et al., 2003; Lippert et al., 2004; Jemima et al., 2014; Mommert et al., 2016). No studies so far have shown that mast cells express histamine H₃ receptors. Given that expression of the histamine H₃ receptor is most commonly associated with the CNS (Schwartz, 2011), this may not be an altogether unexpected finding.

In further studies, the effects of receptor-selective agonists on functional responses were investigated. There was no evidence that any of the agonists selective for a particular histamine receptor were able to induce degranulation or eicosanoid generation from human lung mast cells. Moreover, there was no indication that any of the same histamine agonists could modulate IgE-dependent mediator release. These findings are at odds with studies in human mast cell lines (HMC-1 and LAD2 cells) and cord-blood derived mast cells in which 4-methylhistamine, a putative agonist at histamine H₄ receptors, was able to induce both degranulation and cys-LT generation and these effects of 4-methylhistamine were reversed by the histamine H₄ receptor antagonist JNJ777120 (Jemima et al., 2014). In broader agreement with the present study, H₄ agonists were unable to stimulate degranulation or eicosanoid generation in mouse mast cells (Hofstra et al., 2003). These findings are in stark contrast to studies in human basophils in which a histamine H₄ receptor agonist, ST-1006, inhibited cys-LT generation and other indices of basophil activation (Mommert et al., 2016). The

reasons for these differences in responses among different mast cell types are not immediately apparent. However, the findings highlight the well-recognized functional heterogeneity that exists among mast cells isolated from different species and from sites within the same species (Pearce, 1983; Lowman et al., 1988).

In various models, histamine has been shown to induce chemotaxis. This was first appreciated over 40 years' ago in studies with eosinophils (Clark et al., 1977). At that time, only histamine H₁ and histamine H₂ receptors were recognized and studies with ligands to these receptors indicated that neither histamine H₁ nor histamine H₂ receptors were involved in causing chemotaxis in eosinophils. With the later discovery of the histamine H₃ receptor and studies using ligands to this receptor, the indications were that the histamine H₃ receptor was not involved in eosinophil chemotaxis (Raible et al., 1994). It was only after the histamine H₄ receptor had been identified and probed pharmacologically that it was realized that the histamine H₄ receptor was involved in initiating chemotaxis in eosinophils (Ling et al., 2004) and that the histamine H₂ receptor might be involved in blunting chemotactic responses (Reher et al., 2012).

In addition to eosinophils, histamine has also been shown to recruit other inflammatory cells most notably mast cells. For example, aerosolized histamine in mice has been shown to increase mast cells in tracheal slices and also to induce migration of mast cells to sub-epithelial regions (Thurmond et al., 2004). This redistribution of mast cells by histamine was antagonized by the H₄ antagonist, JNJ777120 (Thurmond et al., 2004). In the present study, histamine induced chemotaxis of human lung mast cells. This effect appeared to be mediated by histamine H₄ receptors for two reasons. First, an H₄-selective agonist, JNJ28610244, was also effective at inducing chemotaxis whereas an H₁-selective agonist, 2-(2-

pyridyl) ethylamine, was not. Second, migration of mast cells induced by the H₄ agonist, JNJ28610244, was effectively prevented by the H₄ antagonist, JNJ7777120. Collectively, these findings provide strong evidence that the histamine H₄ receptor mediates chemotaxis of human lung mast cells.

The extent to which these *in vitro* data might extrapolate to the *in vivo* situation is hard to equate. Quite high concentrations of agonists are needed to promote chemotaxis in the experimental system used in the present study. However, it is quite likely that, in an allergic disease context, quite high concentrations of histamine are generated locally. An additional consideration is whether an experimental chemotaxis chamber model using isolated cells can mimic closely the migration of cells in tissue. If the experimental system used herein is representative, then these data suggest that high, localized concentrations of histamine might mediate mast cell accumulation in the lung. As a corollary, these findings support ongoing initiatives to develop histamine H₄ receptor antagonists for the treatment of asthma and related allergic diseases such as atopic dermatitis (Thurmond et al., 2008; Walter et al., 2011; Liu, 2014; Thurmond, 2015).

In summary, we have shown that engagement of histamine receptors has no effect on mediator release from mast cells. By contrast, activation of histamine H₄ receptors promotes migration of human lung mast cells. This mechanism may contribute to mast cell accumulation in the lung.

Acknowledgements

The authors would like to thank Mr Rao, Miss Socci, Mr Edwards, Mr Hopkinson and Miss Tenconi (Cardiothoracic Surgery, NGH, Sheffield), and Dr Kitsanta and Dr Bury (Histopathology, RHH, Sheffield) for their help in providing lung tissue. The Authors would also like to thank Liz Seward (University of Sheffield) for the provision of LAD2 cDNA and Colin Bingle for providing RNA from breast cancer cell lines. We thank Janssen Research & Development, LLC (San Diego, USA) for the kind gift of JNJ28610244.

Conflict of interest statement

None

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Fig. 1 Histamine receptor expression in human lung mast cells determined by RT-PCR. Isolated RNA was converted to cDNA by reverse transcriptase (+) and, as a control, this step was also carried out in the absence of reverse transcriptase (-). Amplification of cDNA was performed using primers for histamine H₁, histamine H₂, histamine H₃ and histamine H₄ receptors and for β -actin (see Table S1 in Supporting Information Repository for primer details). Expression of histamine receptors for two mast cell preparations (HLMC-1, HLMC-2) is shown as well as for LAD-2 cells. Data are representative of a total of 5 different mast cell preparations in excess of 95% purity. Lanes at either end of each gel represent a 100 bp ladder.

Fig. 2 Histamine receptor expression in human lung mast cells determined by q-PCR. Expression of histamine H₁, histamine H₂, histamine H₃ and histamine H₄ receptors and β -actin was determined in human lung mast cells using primer probes as shown in Table S2 (Supporting Information Repository). Values are means $\pm$ S.E.M. for 7 different mast cell preparations.

Fig. 3 Effects of histamine agonists on mediator release. Mast cells were incubated (15 min) without (-) or with either 2-(2-pyridyl) ethylamine (H₁ agonist), amthamine (H₂ agonist), methimepip (H₃ agonist) and JNJ28610244 (H₄ agonist) before challenge for a further 25 min without or with a half-maximal releasing concentration of anti-IgE (0.2 μ g/ml) for mediator release. Effects of histamine agonists on (A) histamine, (B) cys-LT and (C) PGD₂ generation were evaluated. Values are means $\pm$ S.E.M. for 5 different mast cell preparations.

Fig. 4 Mast cell chemotaxis. Mast cells were placed in the upper chamber and either buffer (control) or chemotactic agent was placed in the lower chamber. As an additional control, chemotactic agent was placed in both the upper and lower chambers (chemokinesis). The effects of (A) SCF (10 ng/ml) and (B) histamine (0.1

– 10 μ M) on chemotaxis were investigated. In (B), chemokinesis was studied at the highest concentration (10 μ M) of histamine. After incubating the chemotaxis plate for 4 h at 37 °C in a CO₂ incubator, the number of mast cells that had migrated into the lower chamber were visualized by microscopy using an alcian blue stain. Values are means $\pm$ S.E.M for (A) 14 and (B) 4 different mast cell preparations. Statistically significant increases in migration compared to control are indicated, * P < 0.05.

Fig. 5 Effects of JNJ28610244 (H₄ agonist) and 2-(2-pyridyl) ethylamine (H₁ agonist) on chemotaxis. Mast cells were placed in the upper chamber and either buffer (control) or chemotactic agent was placed in the lower chamber. As an additional control, chemotactic agent was placed in both the upper and lower chambers (chemokinesis). The effects of (A) JNJ28610244 or (B) 2-(2-pyridyl) ethylamine on mast cell chemotaxis were investigated. Chemokinesis was also assessed at the highest concentration (10 μ M) of agonists. (C) In these experiments, studies with SCF (10 ng/ml) were also performed in parallel as a positive control. After incubating the chemotaxis plate for 4 h at 37 °C in a CO₂ incubator, the number of mast cells that had migrated into the lower chamber were visualized by microscopy using an alcian blue stain. Values are means $\pm$ S.E.M for 5 different mast cell preparations. Statistically significant increases in migration compared to control are indicated, * P < 0.05.

Fig. 6 Effect of JNJ7777120 (H₄ antagonist) on chemotaxis induced by JNJ28610244 (H₄ agonist). Mast cells were placed in the upper chamber and either buffer (control). agonist (10 μ M) and/or antagonist (10 μ M) was placed in the lower chamber. As an additional control, either agonist or antagonist was placed in both the upper and lower chambers to assess chemokinesis. After incubating the chemotaxis plate for 4 h at 37 °C in a CO₂ incubator, the number of mast cells that had migrated into the lower

chamber were visualized by microscopy using an alcian blue stain. Values are means $\pm$ S.E.M for 4 different mast cell preparations. Statistically significant increases in migration compared to control ($*P < 0.05$) and statistically significant antagonism of migration ($^{\#}P < 0.05$) are indicated.

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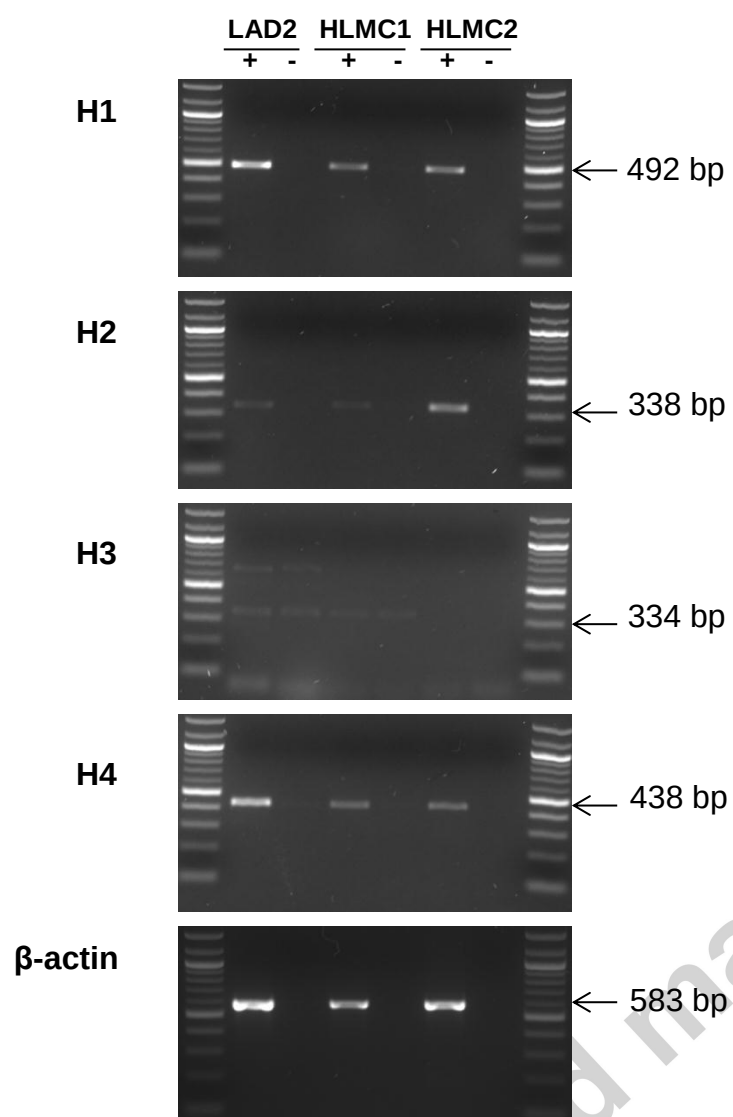


Fig. 1

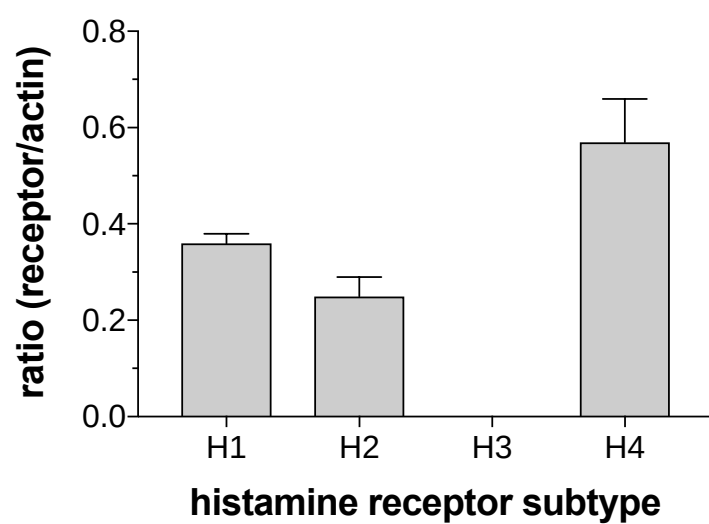


Fig. 2

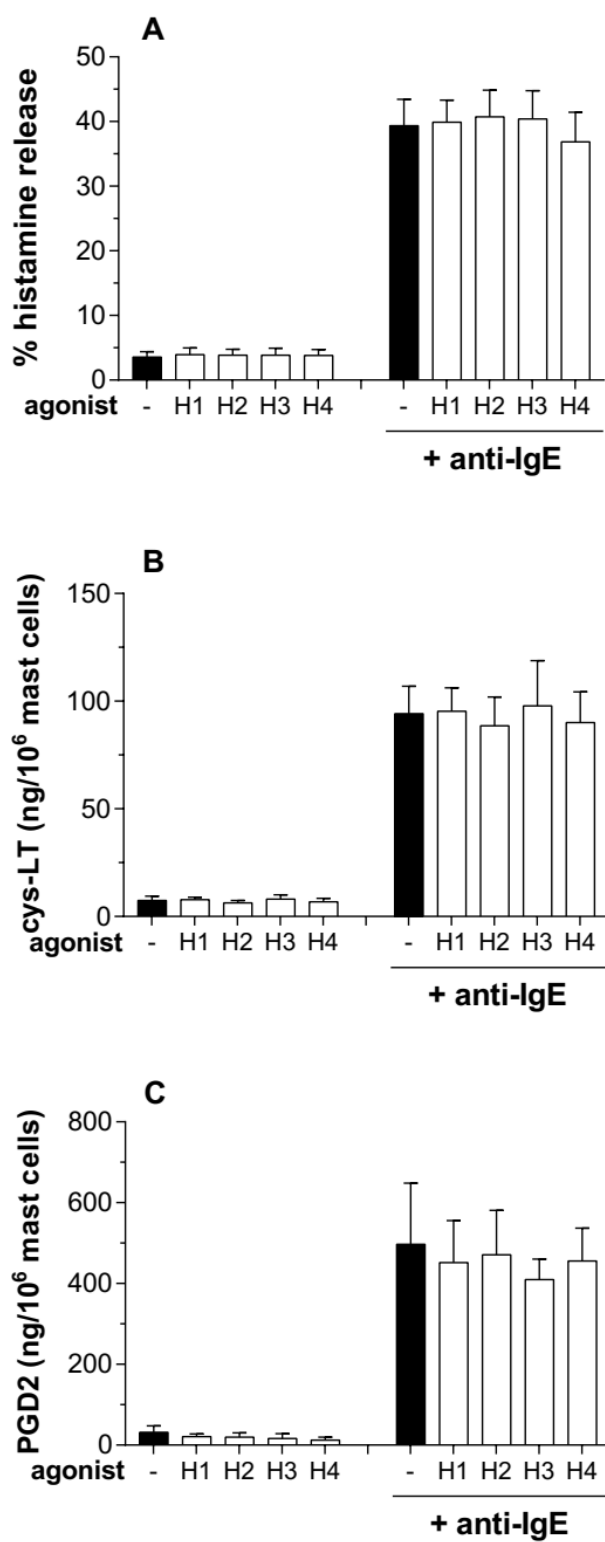


Fig. 3

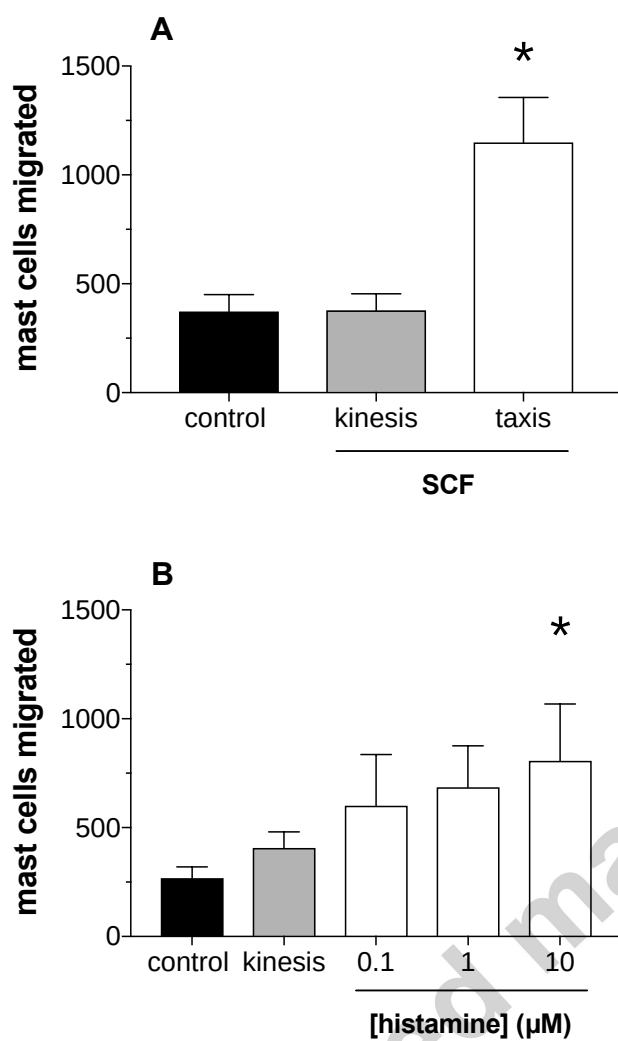


Fig. 4

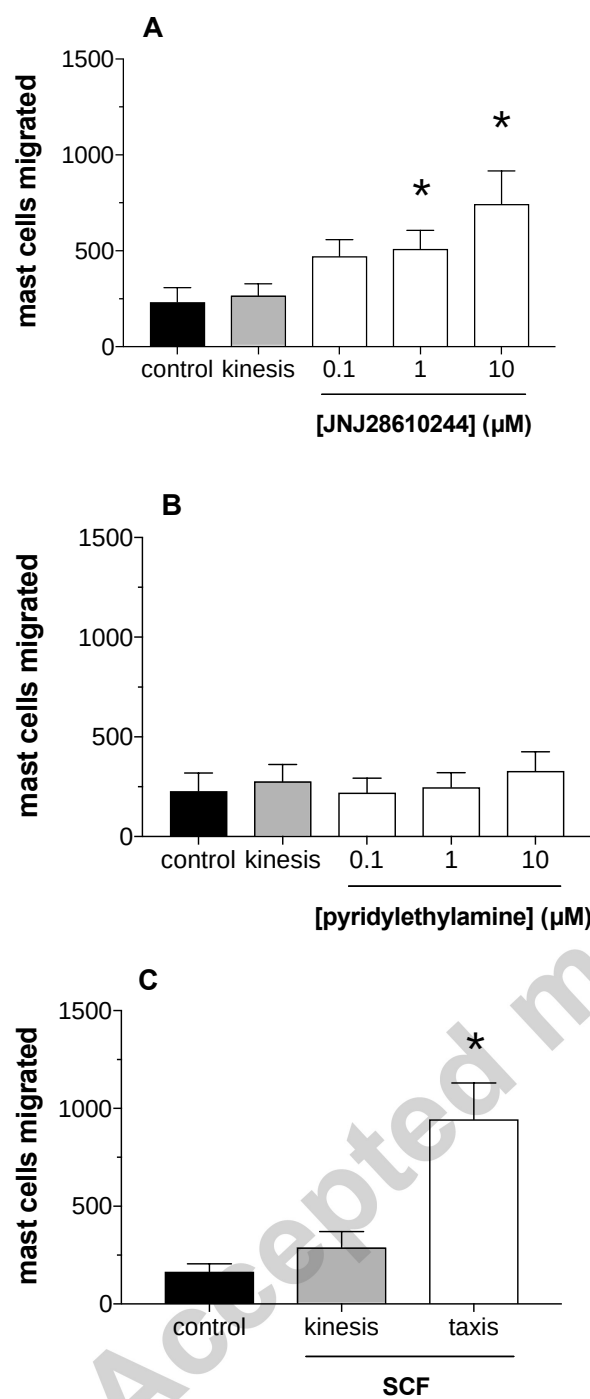


Fig. 5

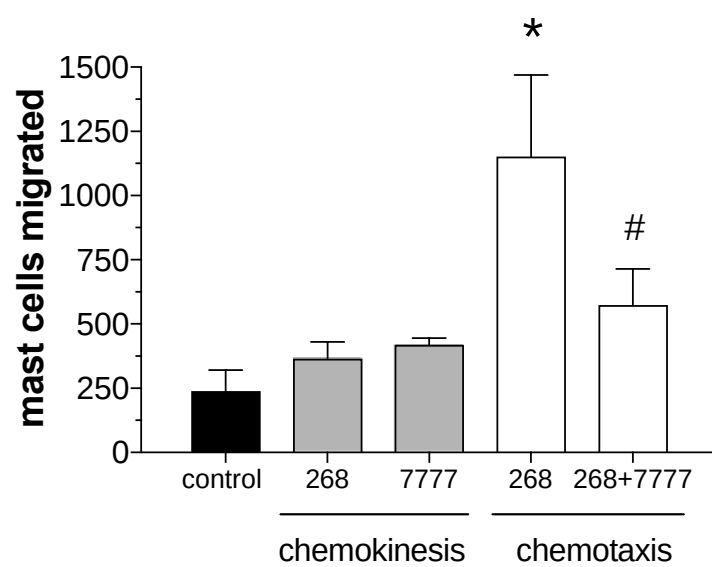


Fig. 6

Supporting Information Repository

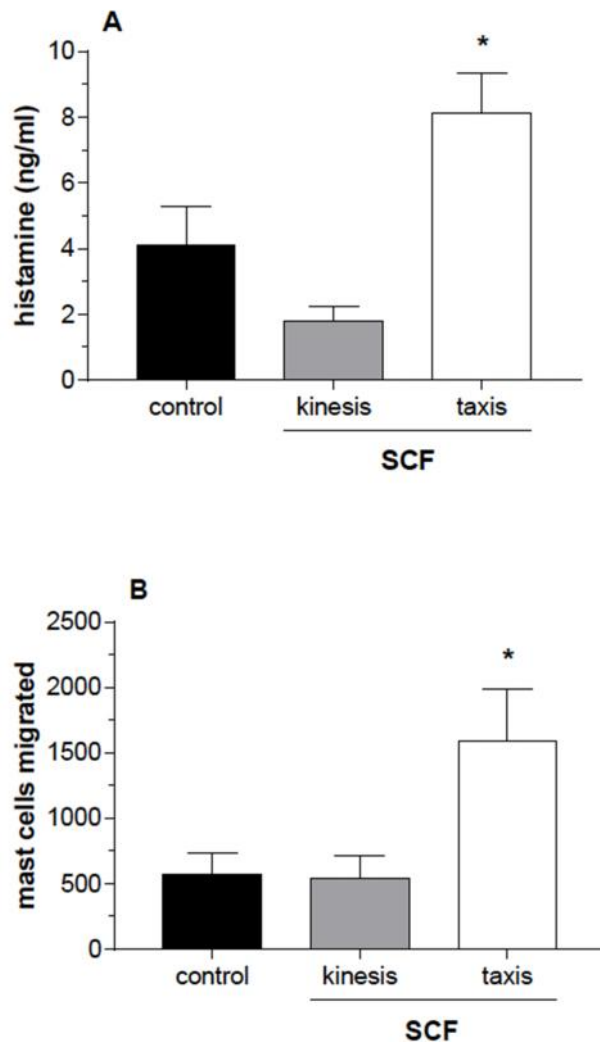


Fig. S1 Assessment of mast cell migration. Mast cells were placed in the upper chamber and either buffer (control) or SCF (10 ng/ml) was placed in the lower chamber. As an additional control, SCF was placed in both the upper and lower chambers (chemokinesis). After incubating the chemotaxis plate for 4 h at 37 °C in a CO₂ incubator, mast cell migration into the lower chamber was assessed by either (A) lysing the cells with perchloric acid (1.6%) and measuring liberated histamine or (B) visualizing mast cells by microscopy using an alcian blue stain. Values are means $\pm$ S.E.M for 5 different mast cell preparations and data in (A) and (B) are paired. Statistically significant increases in migration compared to control are indicated, * $P < 0.05$ at least.

Table S1 Primers used to determine histamine receptor subtype and β -actin expression by RT-PCR

target	primers	annealing conditions	fragment size (bp)
H1R	5'-TAAGCTGAGGCCAGAGAACC-3' (sense) 5'-TACTGTCTTGAATGCGAGCG-3' (antisense)	59° C, 45 s	492
H2R	5'-GACTCTACCGCATGCAAG-3' (sense) 5'-CGCAGTGGGTCCATGAC-3' (antisense)	59° C, 45 s	338
H3R	5'-CGGCTGTCAACCCTGTCCTCTACC-3' (sense) 5'-CCCAGCCTCCAGTCCAGCCAGTGA-3' (antisense)	59° C, 45 s	334
H4R	5'-GGTACATCCTTGCCATCACATCAT-3' (sense) 5'-GAGCCCAGCAAACAGCAAAA-3' (antisense)	59° C, 45 s	438
β -actin	5'-ATATCGCCGCGCTCGTCGTC-3' (sense) 5'-TAGCCGCGCTCGGTGAGGAT-3' (antisense)	60° C, 45 s	583

Table S2 Primer probes used to determine histamine receptor subtype expression by q-PCR

target	primer probe
<i>HRH1</i>	Hs00911670_s1
<i>HRH2</i>	Hs00254569_s1
<i>HRH3</i>	Hs00200610_m1
<i>HRH4</i>	Hs00222094_m1
<i>ACTB</i> (β -actin)	Hs01060665_g