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Molecular determinants of ligand-directed signaling for the histamine H_1 receptor

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Introduction

Histamine activation of the H_1 G protein-coupled receptor (GPCR) predominately activates $G\alpha_q$ protein and stimulation of phospholipase (PL) C to form inositol phosphates (IP) and diacylglycerol (DAG) signaling molecules [1]. This activity can present as respiratory distress, diarrhea, edema, and hypotension associated with an allergic response. In mammalian brain and adrenal gland, H_1 activation also can stimulate $G\alpha_s$ and adenylyl cyclase (AC) to form adenosine 3′,5′-cyclic monophosphate (cAMP), a signaling molecule that modulates catecholamine neurotransmitter synthesis and release [1, 2]. Development of H_1 drugs has focused on antagonists for their anti-allergy effects. Recent understanding of the clinical importance of H_1 receptors in brain, however, suggests pharmacotherapeutic potential of H_1/AC/cAMP agonists in neurodegenerative and neuropsychiatric disorders [3]. Like all mammalian GPCRs except bovine rhodopsin, the 3-dimensional structure of the human histamine H_1 receptor is unknown, compromising rational design of especially agonist H_1 receptor drugs. Here, human H_1 receptor mutagenesis and homology modeling results begin to delineate molecular determinants involved in histamine activation of H_1-mediated PLC/IP/ versus AC/cAMP signaling.

Materials and methods

Histamine competitive displacement of the H_1 radioligand [^{3}H]-mepyramine was measured in membranes prepared from CHO-K1 cells and activity of PLC/IP or AC/cAMP formation was measured in whole CHO-K1 cells transiently expressing human cDNA for wild type (WT), K5.39A, or N5.46A point-mutated H_1 receptors, as previously described [1, 2, 4]. Western blots confirmed receptors were expressed at comparable levels. Data were analyzed by nonlinear regression and expressed as mean percent basal condition. Measurements were in triplicate and experiments repeated using a new batch of transfected cells at least three times. The H_1 receptor model was built using Sybyl 7.2, based on homology to the bovine rhodopsin crystal structure (PDB code 1L9H). Model refinement using molecular dynamics (MD) simulation in a lipid bilayer environment was performed with Gromacs 3.3.1 [5]. Manual docking of histamine to the H_1 model was guided by mutagenesis results. The histamine–H_1 receptor complex was energy minimized and subjected to a 100ps MD

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simulation with C atoms of the receptor constrained (ligand could move freely) using Amber force field and Gasteiger-Huckel charges.

Results and discussion

Results for histamine binding and activation (PLC/IP vs. AC/cAMP signaling) of the human WT H_1 receptor in comparison to K5.39A and N5.46A point-mutated H_1 receptors are summarized in the Table. At K5.39A and N5.46A H_1 receptors, respectively, histamine affinity (K_i) is ~8- and ~40-times lower, and, potency (EC_{50}) to stimulate PLC/IP formation is ~9- and ~320-times lower, but, efficacy (E_{max}) is not significantly ($P > 0.05$) affected, in comparison to WT receptors. Histamine potency and efficacy to stimulate AC/cAMP signaling is not significantly ($P > 0.05$) affected by the K5.39A and N5.46A point mutations. Thus, when H_1 K5.39 and N5.46 are mutated to nonfunctional alanine residues, histamine ability to bind the receptor and activate PLC/IP signaling is compromised, but, histamine ability to activate AC/cAMP signaling is unaffected. These results suggest that K5.39 and N5.46 are important H_1 receptor residues for histamine binding, and, are involved in histamine activation of H_1-mediated PLC/IP signaling, but, not AC/cAMP signaling.

To help understand molecular details involved in agonist activated H_1 signaling for drug design, a 3-dimensional molecular model of the histamine–H_1 receptor complex (Figure) was built that incorporates the mutagenesis results reported above. The model indicates the known ionic interaction between the histamine terminal amine and H_1 D3.32, about 1.7 Å apart, required for binding and activation by histamine [6, 7]. Proposed hydrogen and electrostatic bonding of the histamine imidazole with H_1 residues K5.39 and N5.46, respectively, is consistent with previous studies [8]. Distance of the histamine imidazole unprotonated nitrogen to the H_1 K5.39 protonated nitrogen is 2.1 Å, favorable for H-bonding, and, the histamine imidazole is within 4 Å of the H_1 N5.46 amide, conducive to electrostatic binding.

These studies are the first to report molecular interactions between histamine and the human H_1 receptor that influence PLC/IP vs. AC/cAMP signaling. Information learned here should prove useful to design agonist drugs that modulate brain H_1/AC/cAMP signaling and catecholamine neurotransmission without untoward cardiovascular and respiratory effects associated with activation of H_1/PLC/IP signaling.

References

[1]. Moniri NH, Covington-Strachan D, Booth RG. Ligand-directed functional heterogeneity of histamine H_1 receptors: Novel dual-function ligands selectively activate and block H_1-meditated phospholipase C and adenylyl cyclase signaling. J Pharmacol Exp Ther. 2004; 311:274–81. [PubMed: 15169829]

[2]. Moniri NH, Booth RG. Role of PKA and PKC in histamine H_1 receptor-mediated activation of catecholamine neurotransmitter synthesis. Neurosci Lett. 2006; 407:249–53. [PubMed: 16978782]

[3]. Choksi NY, Nix WB, Wyrick SD, Booth RG. A novel phenylaminotetralin (PAT) recognizes histamine H_1 receptors and stimulates dopamine synthesis in vivo in rat brain. Brain Res. 2000; 852:151–60. [PubMed: 10661506]

[4]. Bruysters M, Pertz HH, Teunissen A, Bakker RA, Gillard M, Chatelain P, et al. Mutational analysis of the histamine H_1-receptor binding pocket of histaprodifens. Eur J Pharmacol. 2004; 487:55–63. [PubMed: 15033376]

[5]. Berendsen HJC, van der Spoel D, van Drunen R. GROMACS: A message-passing parallel molecular dynamics implementation. Comp Phys Comm. 1995; 91:43–56.

[6]. Ohta K, Hayashi H, Mizuguchi H, Kagamiyama H, Fujimoto K, Fukui H. Site-directed mutagenesis of the histamine H_1 receptor: Roles of aspartic acid[107], asparagine[198] and threonine[194]. Biochem Biophys Res Commun. 1994; 203:1096–101. [PubMed: 8093027]

[7]. Ter, Laak AM.; Timmerman, H.; Leurs, R.; Nederkoorn, PHJ.; Smit, MJ.; Den Kelder, GMD. Modelling and mutation studies on the histamine H_1-receptor agonist binding site reveal different binding modes for H_1-agonists: Asp116 (TM3) has a constitutive role in receptor stimulation. J Comput-Aided Mol Des. 1995; 9:319–30. [PubMed: 8523041]

[8]. Jongejan A, Bruysters M, Ballesteros JA, Haaksma E, Bakker RA, Pardo L, et al. Linking agonist binding to histamine H_1 receptor activation. Nat Chem Biol. 2005; 1:98–103. [PubMed: 16408006]

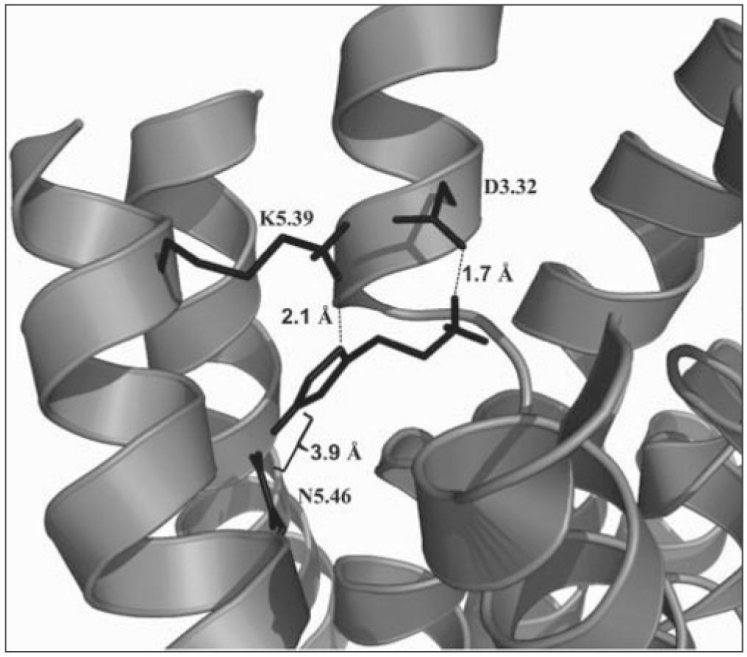

Fig. 1.
Molecular model of histamine (black structure) docked to the human H_1 receptor (relevant side chains are colored black)

Table 1

Histamine Affinity and Functional Activity at H_1 Receptors (statistical comparisons are to WT as P value from *t*-test)

H_1 receptor	**K_i (μM)**	**EC_{50} (μM)/E_{max} (% basal) For formation of**	
		IP	**cAMP**
Wild type	2.99 ± 0.28	0.23 ± 0.06 1100 ± 34	22.7 ± 5.40 140 ± 7.5
K5.39A	22.9 ± 0.19 (P = 0.0001)	2.01 ± 0.01 (P = 0.001) 1500 ± 7.7 %	18.4 ± 0.56 (P = 0.09) 160 ± 10 %
N5.46A	104 ± 16.4 (P = 0.001)	73.3 ± 1.34 (P = 0.003) 1450 ± 12.5 %	15.0 ± 0.13 (P = 0.06) 160 ± 1.6 %