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Different Antagonist Binding Properties of Human and Rat Histamine H_3 Receptors

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Dedicated to Prof. Dr. Dres. h.c. Herbert Oelschläger, Jena, on the occasion of his 80th birthday

Abstract—Different histamine H_3-receptor antagonists have been tested in displacement studies at human and rat H_3 receptors in stably transfected cells. Based on an actual rhodopsin structure, models for receptor–antagonist interaction were developed for receptors of both species. Similarities and discrepancies in binding profiles can be explained, but not quantified by hydrophilic interactions with Asp114 and an important lipophilic binding pocket modified by two nearby amino acids.

Historically the identification of histamine H_3 receptors was performed on rat brain using classical pharmacological methods.[1,2] Later on, its presence in the human brain was confirmed.[3] Due to the large influence of histamine H_3 receptors in different neuroregulatory mechanisms in the brain and in the periphery, a number of therapeutic targets have been proposed (for reviews, see ref 4).

Since the early days of drug development in this field, it has been evident that many compounds showed various potencies with different H_3-receptor test systems. Although this is not astonishing taking into account different test conditions, different assays, and also different species, this led to persistent speculations on H_3 receptor subtypes or other pharmacological properties. The recent cloning of the human,[5] rat,[6,7] and guinea-pig[8] histamine H_3 receptors give new possibilities for comparison of binding properties.

The rat and human histamine H_3 receptors have a 93.5% overall homology and differ by only five amino acids at the level of the transmembrane domain. In agreement with this, there are strong similarities in the affinities of the endogeneous ligand histamine and other H_3-receptor agonists for H_3 receptors of both species. In contrast, however, a discrepancy in binding has been found for some antagonists.[6,7]

For these antagonists, the binding properties of the rat H_3 receptor can be transformed stepwise into human H_3 receptor pharmacology by point mutations (Val122Ala, Ala119Thr) performed in Cos-1 cells using transient expression.[7] It seems clear, however, that these amino acids are not directly involved in the receptor–antagonist interaction. The binding modes of these lipophilic antagonists must differ from those of basic ligands like the agonists or clobenpropit which are presumed to interact more effectively with Asp114, which is highly conserved in all biogenic amine receptors.

Highly potent antagonists with structural similarities for ease of comparison, but also having some structural diversity suitable for a molecular modeling study, have been investigated for their binding to histamine H_3 receptors of both species.

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Chemistry

All compounds were prepared according to known literature methods. In brief, aromatic ether derivatives were prepared from *N*-trityl-protected ω-(1*H*-imidazol-4-yl)alkanol and the corresponding phenol using the Mitsunobu protocol and following acidolysis.[9,10] Aliphatic ethers were obtained by Williamson reaction of imidazole-protected ω-(1*H*-imidazol-4-yl)alkanolate and the corresponding benzyl halide.[11,12] The sulfanyl derivatives were prepared accordingly using imidazole-protected 4-(ω-chloroalkyl)-1*H*-imidazoles and 4-sulfidoacetophenone.[10] The phenylbutane derivative **11** was prepared by Wittig reaction between (1-trityl-1*H*-imidazol-4-yl)methanal and triphenyl-(3-phenylpropyl)-phosphonium bromide followed by acidolysis and catalytic hydrogenation.[13]

Molecular Modeling

In order to elucidate the mode of histamine H_3 receptor–ligand interaction at the molecular level, models of the rat and human receptor were constructed on the basis of a very recently published X-ray structure of bovine rhodopsin.[15] The amino acid sequences of the histamine H_3 receptors were aligned on the rhodopsin sequence based on the general GPCR alignment published by Baldwin et al.[16] Molecular modeling was performed using the BIOPPLYMER module within SYBYL6.6.[17] The generated receptor models were optimized using the AMBER all atom force field as implemented in SYBYL6.6. Molecular dynamics simulations at 310 K were carried out on both receptors in order to relax their structure. Program PROCHECK[18] was used to evaluate the modeled receptor structures. In a first step, the histamine molecule was manually docked into the binding pocket comprising of helices 3–7 using the conserved Asp114 on helix 3 and Glu206 on helix 5 as a guide to the initial orientation. In a second step the 13 ligands shown in Table 1 were automatically docked in both models using the FlexX program.[19] Standard parameters of the FlexX program as implemented in the 6.62 release of the SYBYL package were used for iterative growing and subsequent scoring of the molecules. All amino acid residues within 10 Å of the histamine position in the corresponding complex were used to define the pocket for the FlexX docking. All ligands were considered flexible during the docking run. Only the top solution for each ligand was retained and further stored in a single mol2 file. After the docking procedures, the obtained ligand–receptor complexes were energy minimized using the TRIPOS force field.

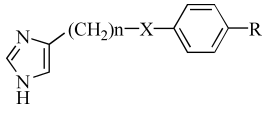
Pharmacology

Clones with stable expression of each histamine H_3-receptor protein were obtained in CHO-K1 cells[7] and assayed in displacement studies. In brief, aliquots of each membrane suspension containing 5–15 μg protein were incubated with 25 pM [^{125}I]iodoproxyfan alone or together with antagonists (200 μL final volume).[7,12b] IC_{50} values were determined using an iterative least-squares method,[20] and K_i values were calculated using the Cheng–Prussoff equation.[21]

Results and Discussion

As expected, all compounds displayed affinities in the nanomolar concentration range for rat and human histamine H_3 receptors (Table 1). This is in accordance with data previously obtained in functional in vitro and in vivo assays of rat, mouse and/or guinea pig.[9–13] Most compounds showed a preference for the rat histamine H_3 receptor compared to their affinities for the human receptor (**1**–**4**, **7**, **8**, thioperamide), whereas proxyfan (**9**) and clobenpropit were equi-effective at both receptors. Compounds **5**, **6**, **10**, and **11** were more potent at the human than at the rat H_3 receptor.

Table 1. Inhibition of [^{125}I]iodoproxyfan binding of antagonists at rat and human histamine H_3 receptors stably expressed in CHO cells[a]



Compounds	*n*	X	R	Rat H_3 $K_i \pm$SEM (nM)	Human H_3 $K_i \pm$SEM (nM)	~Ratios K_i rat H_3: K_i human H_3
1[9,10]	2	O	–CO–CH_3	3.6±0.2	42±1	1:12
2[9]	2	S	–CO–CH_3	0.11±0.02	1.1±0.2	1:10
3[10]	3	O	–CO–CH_3	29±5	87±7	1:3
4[10]	3	O	–CO–cyclopropyl	3.9±0.2[7]	46±4[7]	1:12
5[9]	3	S	–CO–CH_3	44±3	18±1	2:1
6[9]	4	O	–CO–CH_3	36±1	19±1	2:1
7[10]	3	O	–O–CH_3	7.9±0.1	47±3	1:6
8[10]	3	O	H	4.9±0.8	27±4	1:6
9[11]	3	O–CH_2	H	2.9±1[7]	2.7±1[7]	1:1
10[12]	3	O–CH_2	[^{125}I]I	71±4[7]	41±6[7]	2:1
11[13]	3	CH_2	H	12±1[7]	2.1±0.2[7]	6:1
Clobenpropit[14]				1.4±0.1[7]	2.4±0.6[7]	1:2
Thioperamide[2]				6.5±0.2[7]	60±12[7]	1:9

[a]Reference citations given (Ref 9 refers to methods for preparation mentioned therein).

A slight tendency is seen for a preference of the rat receptor for compounds with shorter chain length between the imidazole and the benzene ring whereas compounds with longer chains either show no or a small preference for the human H_3 receptor. As compound **11** without any polar group in the relatively short side chain has substantially higher preference for the human receptor and as molecular modeling studies could not confirm the variations in distances in relationship to the species preference (results not shown), this aspect needs further investigation.

The generated computational models of the human and the rat histamine H_3 receptors (Figs. 1 and 2) show close resemblance to the crystal structure of rhodopsin. As observed for rhodopsin the H_3 receptor is stabilized by an intramolecular disulfide bridge between Cys188 and Cys107. The disulfide bridge is also responsible for the deep folding of the extracellular loop (between helix 4 and helix 5) into the binding pocket. It seems that this loop acts as a barrier to prevent the ligands from moving freely to the extracellular space.

The modeled receptor structures were then used for the docking of the 13 investigated ligands. The results of the docking allowed the identification of key features of the ligands that are responsible for their potencies.

General features of the present models are as follows: The imidazole ring of the ligands is located in a polar pocket formed by several residues capable of forming hydrogen bonds [Tyr115, Glu191, Glu206 as well as Ser203 and Thr375 (not shown)]. Glu206 located on helix 5 and Glu191 located on a loop which folds deeply in the binding pocket make hydrogen bond interactions to the imidazole ring (not shown). The aromatic system of Tyr115 (helix 3) further stabilizes the imidazole ring in this position. Interestingly, a comparable mode of interaction has recently been found for histamine bound to female-specific histamine binding protein (1QFT).[22] The tautomerism of the imidazole ring allows the molecules to interact with both glutamate residues.

Several aromatic and aliphatic amino acid residues constitute the hydrophobic part of the binding pocket (Tyr91, Trp110, Leu111, Cys188, Phe398, and Trp402) (Figs. 1 and 2). They interact with the aliphatic chains and the aromatic systems of the antagonists. The hydrophobic substituents on the aromatic ring system of compounds **1**–**7** point to a hydrophobic cleft between helix 2 and helix 7 formed by the three aromatic residues Phe398, Tyr91, and Trp110. Glu206 on the one site and Tyr91 (helix 2) and Cys188 (which is part of the intramolecular disulfide bridge) on the other site constitute the terminal ends of the lipophilic binding pocket.

Asp114 on helix 3, which is the counterpart for the protonated nitrogen of histamine H_3-receptor agonists,[23] makes no direct hydrogen bond to any of the investigated ligands thereby interacting with the SH group of Cys87 (Figs. 1 and 2). However, the side chain of Asp114 is flexible allowing the carboxylate group to interact with the polar isothiourea group of clobenpropit (due to the bulky cyclohexyl ring of thioperamide its related thiourea group is not accessible for Asp114). Since up to now we have performed our docking studies only with the rigid receptor protein, the influence of this type of interaction cannot be calculated quantitatively by the current models. Further molecular dynamics simulations are in progress for a more detailed description of this type of interaction.

The global orientation of the antagonists was found to be very similar in both receptor models and is related to the relatively small differences in each binding profile. The orientation is comparable to the one of the retinal molecule found in the rhodopsin X-ray structure.[15] The only differences between both receptors showing an influence on the binding pocket are residues 119 and 122

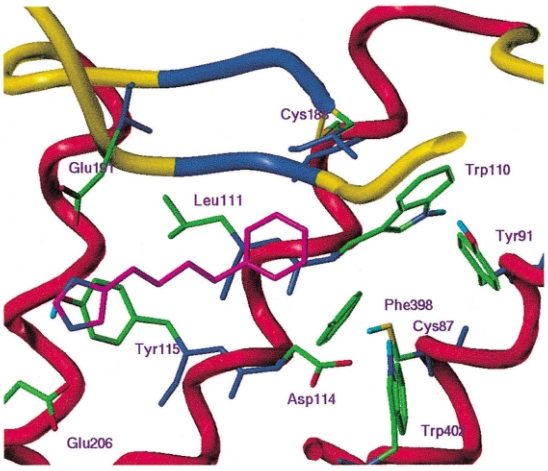


Figure 1. Possible interaction sites between compound **11** (magenta) and the human H_3 receptor are shown (helices in front of the ligand are not displayed for reasons of clarity).

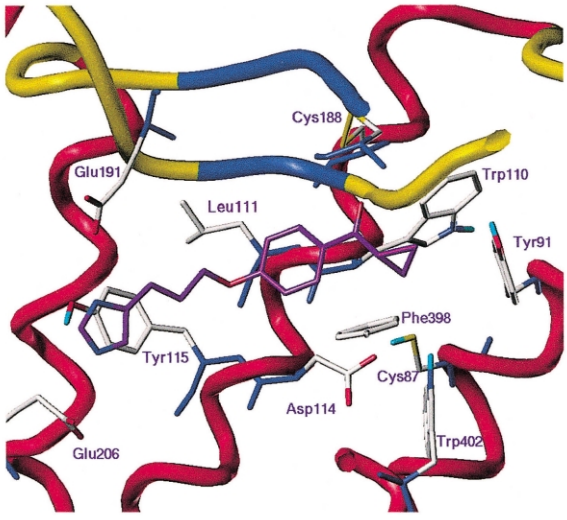


Figure 2. Possible interaction sites between ciproxifan (**4**, violet) and the rat H_3 receptor are shown (helices in front of the ligand are not displayed for reasons of clarity).

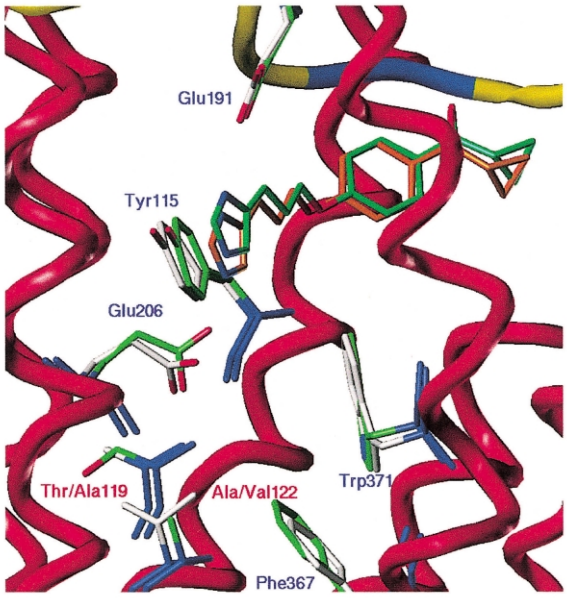


Figure 3. Influence of amino acids Thr/Ala119 and Ala/Val122 on the binding site and on the binding of ciproxifan (**4**) (rat H_3 receptor residues are colored white and bound ciproxifan orange, human H_3 receptor residues are colored green and bound ciproxifan green).

located on helix 3. These two residues (Thr119 and Ala122 in the case of the human receptor, Ala119 and Val122 in the case of the rat receptor) are located in close proximity to Glu206 and Tyr115, thereby influencing the geometry of this polar binding pocket (Fig. 3). Both residues have been shown to be important for the selectivity profile of the investigated compounds.[7] Due to repulsion between Val122 and the two aromatic residues Trp371 and Phe367, this part of the binding pocket shows differences between rat and human H_3 receptors. Since the investigated non-protonated compounds make no or, in the case of clobenpropit, only a potential interaction with Asp114 at helix 3, the modified geometry of the binding pocket influences the interaction with the hydrophobic and aromatic residues of the receptor (Fig. 3). This leads to a modified binding profile of some of the investigated compounds. The quantification of the different lipophilic interactions of both receptors is extremely difficult to calculate since they are the sum of small differences changing in each receptor–ligand conformation (cf. Fig. 3). Further investigations are in progress for a more detailed description of these factors. In the case of protonated agonists, the strong interaction between the positively charged nitrogen and the aspartic acid may compensate these differences, resulting in a similar binding profile at human and rat H_3 receptors.

In conclusion, differences and similarities in the binding profiles at rat and human histamine H_3 receptors of different antagonists can be explained, but not quantified by their receptor–ligand interactions based on computational models. The importance of the lipophilic binding pocket at the human H_3 receptor compared to that of the rat makes these models useful for the prediction of species differences and for further drug design optimized for human therapeutic applications.

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