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REVIEW

Molecular and biochemical pharmacology of the histamine H_4 receptor

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The elucidation of the human genome has had a major impact on histamine receptor research. The identification of the human H_4 receptor by several groups has been instrumental for a new appreciation of the role of histamine in the modulation of immune function. In this review, we summarize the historical developments and the molecular and biochemical pharmacology of the H_4 receptor.
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Keywords: histamine H_4 receptor; molecular pharmacology; biochemistry; signal transduction; species difference; isoforms; heterogeneity; selective agonists and antagonists

Abbreviations: cDNA, complementary DNA; EL2, second extracellular loop; GPCR, G protein-coupled receptor; PTX, pertussis toxin; SNP, single nucleotide polymorphism; TM, transmembrane domain

Introduction

Histamine is known since long to be an important mediator in different (patho)physiological conditions. In 1966, Ash and Schild suggested the presence of two distinct histamine receptor subtypes (Ash and Schild, 1966) based on the pharmacological effects of known histaminergic ligands on, for example, airway smooth muscle (H_1) and the heart, uterus and stomach (H_2). Sir James Black *et al.* at SK&F paved the way for the final acceptance of two distinct histamine receptor subtypes with the development of the first selective histamine H_2 antagonists metiamide and burimamide (Black *et al.*, 1972). Moreover, the recognition that different actions of histamine could be effectively inhibited by selective receptor antagonist eventually resulted in the development of various blockbuster H_1 and H_2 antagonists.

In 1983 a new histamine H_3 receptor subtype was identified by Arrang *et al.* in the rat brain on the basis of classical pharmacology (Arrang *et al.*, 1983). For a long time, this new histamine receptor subtype was not fully endorsed by the pharmaceutical industry because of the lack of molecular details of the receptor protein. While the gene for the histamine H_1 (Yamashita *et al.*, 1991) and H_2 receptor (Gantz *et al.*, 1991) were both cloned in 1991, for many years no definite proof of the molecular architecture of the H_3 receptor was available. Finally, in 1999 a team of J&J researchers led by Tim Lovenberg cloned the complementary DNA (cDNA) of an α_2 adrenergic-like EST sequence that, after characterization, turned out to encode for the human histamine H_3 receptor (Lovenberg *et al.*, 1999). This cloning of the H_3 receptor gene resulted in a rapid increase in knowledge of its molecular pharmacology and biochemistry (e.g. expression, signal transduction, receptor isoforms) (Lovenberg *et al.*, 1999; Drutel *et al.*, 2001; Hancock, 2006; Bongers *et al.*, 2007). Combined with the development of recombinant cell systems, these developments led to a huge interest from pharmaceutical companies (Leurs *et al.*, 2000). Many new selective ligands have since then been reported in (patent) literature (Celanire *et al.*, 2005; Wijtmans *et al.*, 2007), and the H_3 receptor is currently seen as a new target for the treatment of a variety of central nervous system disorders (e.g. attention deficit hyperactivity disorder, Alzheimer's disease and schizophrenia) (Leurs *et al.*, 1995; Esbenshade *et al.*, 2008).

The cloning of the H_3 receptor gene also marked the entry of the histamine H_4 receptor into the G protein-coupled receptor (GPCR) family of histamine receptors. Using the DNA sequence of the histamine H_3 receptor, several research groups independently identified a previously unexplored GPCR sequence in the human genome as a new histamine H_4 receptor (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001). The new receptor showed a distinct pharmacology (see

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below) is highly expressed in immune cells (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001) and is now regarded as an interesting target for, for example, inflammatory disorders and pruritis (de Esch *et al.*, 2005; Thurmond *et al.*, 2008). The first identification of the H_4 receptor led to a further increase in the interest in histamine receptor pharmacology and a substantial rise in H_4 receptor-related papers (112 in the period 2001–2009) and patents (36 in the period 2002–2008) in the last few years. In the present review we will highlight the molecular biology and pharmacology of the latest member of the histamine receptor family.

Cloning of the histamine H_4 receptor gene

As reported above, the H_4 receptor cDNA was finally identified in the human genome database on the basis of its overall homology (37%) to the H_3 receptor sequence (Nakamura *et al.*, 2000; Oda *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001). The homology of the H_4 receptor to the H_1 and H_2 receptor is only ~19%, explaining why this subtype was only found after the long awaited identification of the H_3 receptor gene in 1999 (Lovenberg *et al.*, 1999). The size of the open reading frame of the H_4 receptor cDNA is 1173 bp, encoding for a 390 amino acid protein (Figure 1), belonging to the large family of GPCRs. Besides a high sequence homology, the H_4 receptor also shares its gene structure with the H_3 receptor (Coge *et al.*, 2001a; Coge *et al.*, 2001b). The human H_4 receptor gene is present in a single copy on chromosome 18q11.2. The gene spans 16.98 kb (position 20 294 591 to 20 311 567) and, like the H_3 receptor (Drutel *et al.*, 2001), is interrupted by two large introns (7867 bp and >17 500 bp). The introns divide the coding regions into three parts, encoding amino acid number 1–65, 66–119 and 120–390. Such an intron–exon distribution has led for the H_3 receptor to the generation of a large number of alternatively spliced GPCR variants, sometimes with clearly altered functionalities (Drutel *et al.*, 2001; Leurs *et al.*, 2005). However, for the H_4 receptor at present only two non-signalling, non-7TM H_4 receptor isoforms have been identified (van Rijn *et al.*, 2008) (Figure 1), designated as $H_4(67)$ and $H_4(302)$. The polypeptide of $H_4(67)$ is prematurely terminated at residue 67, while $H_4(302)$ lacks residues 68–155 (Figure 1, *vide infra*) (van Rijn *et al.*, 2008).

The original H_4 receptor amino acid sequence as reported by Oda *et al.* (2000) deviates at three positions from the sequences reported by four other research groups (Nakamura *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Zhu *et al.*, 2001), that is, Val instead of Ala at position 138, Arg instead of His at position 206 and Arg instead of Gln at position 253. The $Val^{138}Ala$ and $Arg^{206}His$ polymorphisms have been confirmed by single nucleotide polymorphism (SNP) analysis involving samples from White, Chinese, Japanese, Afro-

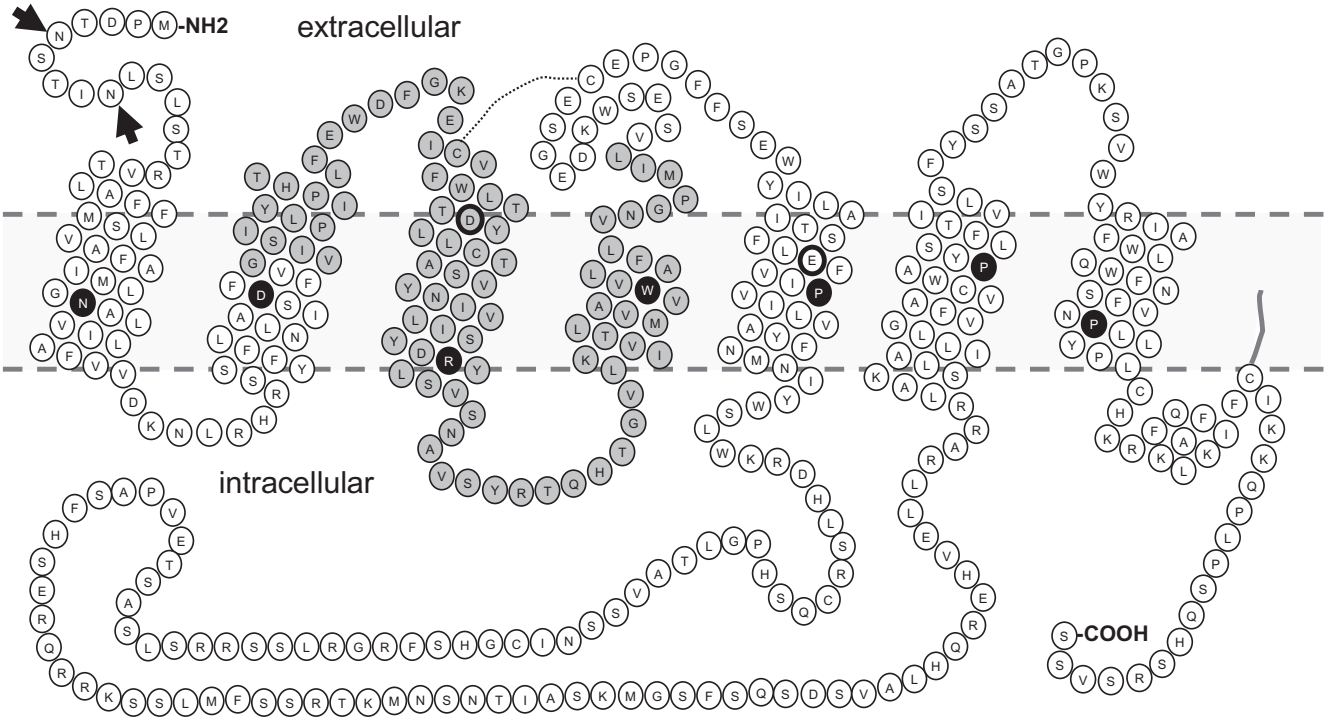

Figure 1 Snake plot of the human histamine H_4 receptor. The full-length receptor consists of 390 amino acids, which form seven transmembrane helices, three extracellular loops and three intracellular loops, with an extracellular N-terminal and an intracellular C-terminal peptide; the dotted line represents the putative disulfide bridge that links the cysteines in the third transmembrane and second extracellular loop. Residues $Asp^{3.32}$ and $Glu^{5.46}$ that play important roles in histamine binding are marked with bold border. The H_4R_{67} isoform only contains the first 67 N-terminal residues (marked in white), while the H_4R_{302} lacks the residues marked in grey. The conserved residues in the family A of G protein-coupled receptors are depicted in black circles, while the putative glycosylation sites (Asn^5 and Asn^9) are indicated with arrows. A potential palmitoylation site (Cys^{374}) at the C-terminal tail is suggested to be close to the membrane following membrane insertion of a putative attached palmitic acid.

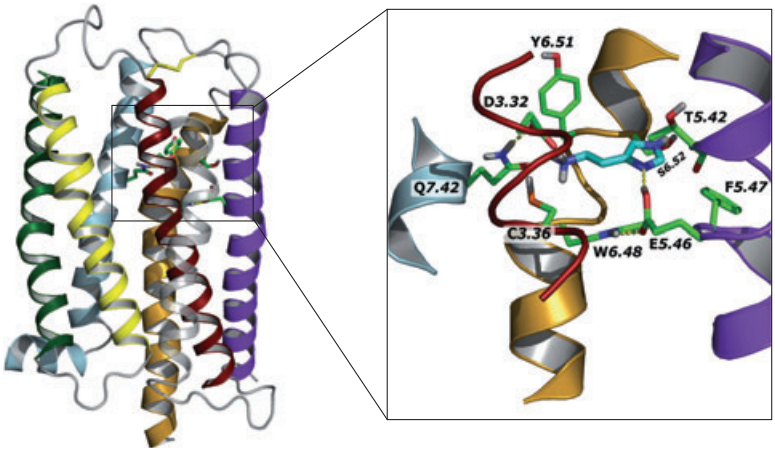

Figure 2 Schematic representation of a homology model of the human histamine H_4 receptor. The figure shows the seven transmembrane domains and the position of the ligand binding site. In the box details of the histamine binding site, as determined using *ab initio* calculations is shown (reproduced with permission from Jongejan *et al.*, 2008).

American and sub-Saharan African people (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=59340). The occurrence of Ala^{138} or His^{206} alleles is higher than that of Val^{138} or Arg^{206} (91.1–100% vs. 0–9.2%). Two other SNPs have been found as well in the coding region, resulting in $Cys^{284}Ser$ mutation and a frame shift at residue L^{379}. Besides those, 37 SNPs are found in intron 1, 45 in intron 2 and 21 in 3′-untranslated region (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=59340). So far no functional comparisons of the various SNP variants or linkage studies have been reported.

As with the other histamine receptor family members, the H_4 receptor protein possesses all consensus motifs identified for the Class A rhodopsin-like GPCRs, that is, $N1.50^{33}$, $D2.50^{61}$, $R3.50^{112}$, $W4.50^{140}$, $P5.50^{186}$, $P6.50^{318}$ and $P7.50^{355}$ [using the standardized GPCR nomenclature according to Ballesteros–Weinstein (Ballesteros and Weinstein, 1995) (Figure 1)]. With respect to histamine binding, a conserved aspartate residue in the third transmembrane (TM) aminergic GPCRs helix (D3.32, Figure 1) is also present in this newest member of the aminergic GPCR family. As for the other aminergic GPCRs, D3.32 is believed to interact with the protonated amine group of the agonist histamine (Shin *et al.*, 2002). Moreover, in both H_3 and H_4 receptors a second negatively charged amino acid, E5.46, is present in the fifth TM helix (Figure 1). This amino acid has been shown to play an essential role in the binding of histamine as determined by site-directed mutagenesis analysis and computational analysis (Shin *et al.*, 2002; Uveges *et al.*, 2002). As is illustrated in Figure 2, E5.46 is a key anchor for the imidazole ring of histamine. The binding pocket for the imidazole ring is formed by E5.46, T5.42 and W6.48 (Jongejan *et al.*, 2008). The latter residue adopts the 'active' rotamer conformation (Jongejan *et al.*, 2005) by forming a hydrogen bond to the aforementioned E5.46 (box, Figure 2).

The H_4R has two potential glycosylation sites (Asn^5 and Asn^9) (Figure 1, *vide infra*), whereas Cys^{374} might serve as a potential site for palmitoylation. The H_4R also contains cysteine residues that potentially form a disulphide bridge, connecting the third TM with the second extracellular loop (EL2) (Figure 1). These cysteine residues are conserved among the GPCRs, and the disulphide bridge has been evidenced in the crystal structure of bovine rhodopsin and adrenergic β_1 and β_2 receptors (Palczewski *et al.*, 2000; Cherezov *et al.*, 2007; Warne *et al.*, 2008). The disulfide bridge will bring some structural constraint to the extracellular loop two, which may be important in view of the suggested involvement of the EL2 in the binding of histamine (Lim *et al.*, 2008).

Species differences

Following the identification of the human H_4R, the cDNA sequence of mouse, rat, guinea pig, pig, dog and monkey H_4 receptors have been reported and functionally expressed (Liu *et al.*, 2001b; Oda *et al.*, 2002; 2005). The monkey H_4 receptor (*Macaca fascicularis*) shows a high sequence homology (92%) with the human orthologue (Oda *et al.*, 2005). The H_4 receptors of the other species are only moderately homologous to the human H_4R, with sequence homology between 67% and 72% (Liu *et al.*, 2001b; Oda *et al.*, 2002; 2005; Jiang *et al.*, 2008). Forty (partial) amino acid sequences of H_4R orthologues from 34 other species have been extracted from various genomic ENSEMBL databases (http://www.ensembl.org/Homo_sapiens/Gene/Compara_Ortholog?db=core;g=ENSG00000134489;r=18:20294591-20313918;t=ENST00000256906), among others horse (*Equus caballus*), cow (*Bos taurus*), cat (*Felis catus*), rhesus monkey (*Macaca mullata*), chimpanzee (*Pan troglodytes*) and dolphin (*Tursiops truncates*) H_4 receptor sequences are known (Figure 3). The H_4R sequences from cynomogus (*Macaca fascicularis*) and pig (*Sus scrofa*), which are not listed as orthologues in the ENSEMBL database are also included in the phylogenetic tree in Figure 3. Because some of the sequences are still incomplete, changes in the phylogenetic tree are to be expected. Fish (zebra fish, fugu, tetraodon, medaka and stickleback) as well as frog (*Xenopus tropicalis*) have each two orthologues. However, these orthologues, together with the sequences from chicken (*Gallus gallus*) and opossum, show only 28–42% homology to the human H_4 receptor, while all the others show a substantial higher

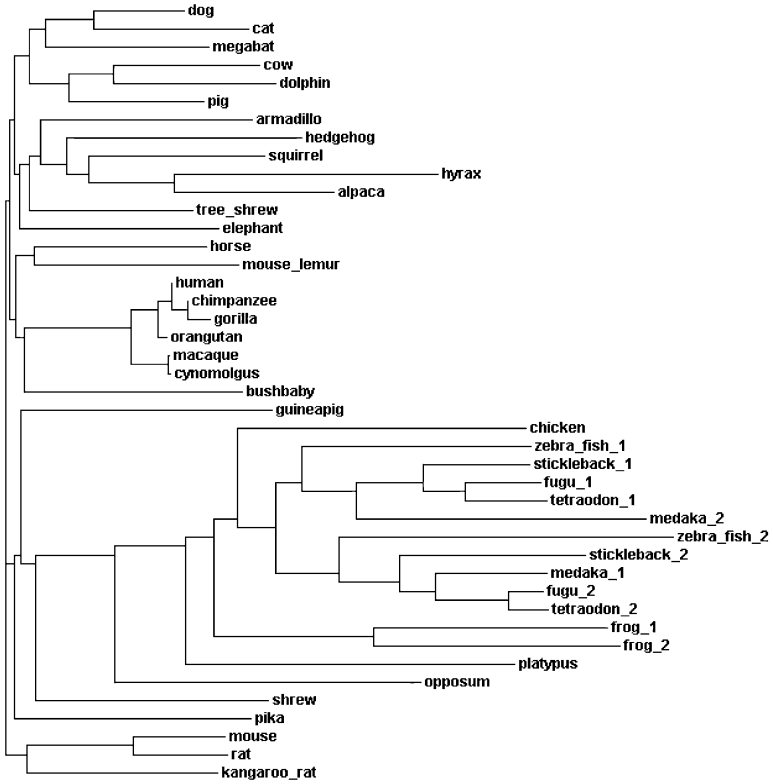

Figure 3 Phylogenetic tree of H_4 receptor orthologues. The sequences are obtained from http://www.ensembl.org and the phylogram was created with ClustalW.

homology to the human H_4 receptor (>50%). In fact, these orthologues are more similar to the human H_3R. Consequently, in the phylogenetic tree these H_3/H_4 orthologues cluster in a separate group (Figure 3). Within the extracted (partial) GPCR sequences, the typical aminergic GPCR features mentioned earlier (e.g. $Asp^{3.32}$ in TM3 and $Glu^{5.46}$ in TM5) can often be found. Detailed analysis of most of these species variants is for the moment however lacking, but may provide useful tools to dissect receptor–ligand binding. Previously, Lim *et al.* (2008), for example, showed that a sequence difference in the extracellular loop EL2 is responsible for the lower affinity of histamine for rat and mouse H_4 receptors. Comparing the binding profiles of rat, mouse, guinea-pig, porcine, monkey and dog H_4 receptors, one can identify for ligands like clozapine and clobenpropit (see also paragraph on H_4 agonist) also substantial differences in binding affinity between the various species (Liu *et al.*, 2001b; Oda *et al.*, 2002; 2005). Differences in pharmacological activities of H_4 receptor ligands between the different species might hamper preclinical development of future H_4 receptor drugs.

Signal transduction of the H_4 receptor

The H_4 receptor is coupled to pertussis toxin (PTX)-sensitive $G\alpha_{i/o}$ proteins, thereby inhibiting forskolin-induced cAMP and eventually the modulation of transcription of genes regulated by cAMP-responsive elements in cell lines recombinantly expressing the H_4 receptor (Oda *et al.*, 2000; Liu *et al.*, 2001a; Zhu *et al.*, 2001). In transfected HEK-293 cells H_4 receptor stimulation also leads to a strong increase in mitogen-activated protein kinase phosphorylation via a PTX-sensitive pathway (Morse *et al.*, 2001). Furthermore, the H_4 receptor also signals to calcium via either PTX-sensitive $G\alpha_{i/o}$ proteins (Hofstra *et al.*, 2003) or the promiscious $G\alpha_{16}$ protein (Oda *et al.*, 2000; Morse *et al.*, 2001), which is selectively expressed in immune cells (Wilkie *et al.*, 1991). In recombinant cell lines the H_4 receptor constitutively inhibits forskolin-induced cAMP-responsive element-mediated reporter gene expression and activates [^{35}S]GTPγS binding (Liu *et al.*, 2001a; Morse *et al.*, 2001; Lim *et al.*, 2005). The agonist-independent activity can be inhibited by H_4 inverse agonists, such as thioperamide.

Activation of endogenously expressed H_4 receptors in for example eosinophils and mast cells leads to PTX-sensitive calcium mobilization (Buckland *et al.*, 2003; Hofstra *et al.*, 2003), via the activation of phospholipase C (Hofstra *et al.*, 2003). The increased cytosolic calcium concentration is intimately linked to actin polymerization, cell shape change and eventually to the migration of mast cells, eosinophils and monocyte dendritic cells (Buckland *et al.*, 2003; Hofstra *et al.*, 2003; Ling *et al.*, 2004; Gutzmer *et al.*, 2005; Barnard *et al.*, 2008).

Looking back in immunology literature with this new knowledge, early examples of H_4 receptor-mediated signalling by histamine can be identified. Already in the 1975 Clark *et al.* observed histamine-induced eosinophil chemotaxis that could not be blocked by the known H_1 and H_2 receptor antagonists (Clark *et al.*, 1975). Furthermore, in 1994 Raible *et al.* identified in eosinophils a PTX-sensitive cytosolic

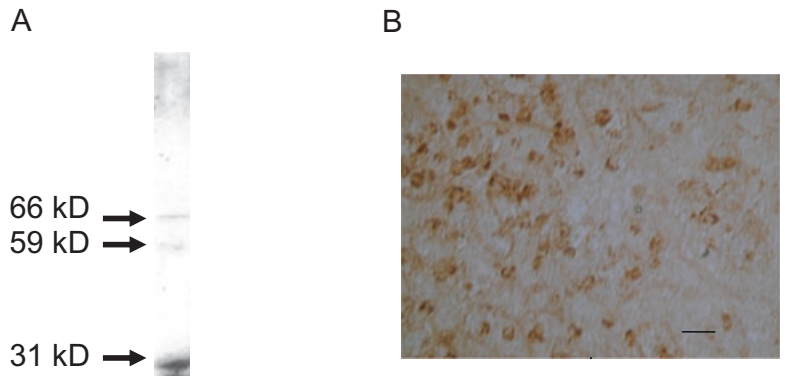

Figure 4 Anti-h$H_{4(374–390)}$ receptor antibody reacts with human H_4 receptor protein in spleen tissue lysate and sections. (A) Immunoblot of human spleen lysate (10 μg). Anti-$H_{4(374–390)}$ antibodies were used at 2 μg·mL^{-1}, incubated overnight at 4°C and developed as described in van Rijn *et al.* (2006). In addition to the putative monomer at M_w 31 kDa, two higher molecular weight species were detected at 59 and 66 kDa. (B) Immunostaining of human spleen slice (bar = 50 μm) using anti-$H_{4(374–390)}$ at 1 μg·mL^{-1} using an immunohistochemical protocol described in Chazot *et al.* (2001).

calcium increase induced by histamine, which could be inhibited by the presumed selective H_3 receptor antagonist thioperamide. The potent H_3 receptor reference agonist *R*-α-methylhistamine also induced a calcium increase, but at much lower potency than that of histamine itself. Based on these observations, the presence of a new histamine receptor subtype was suggested (Raible *et al.*, 1994). Alas, this suggestion was largely ignored by the histamine research community and has only been fully exploited more than a decade after the discovery.

Anatomical Topography of the H_4 receptor

The tissue distribution of the H_4 receptor has been profiled at the mRNA level in a wide range of human (using reverse transcription polymerase chain reaction) and mouse (using *in situ* hybridization) tissues (Zhu *et al.*, 2001). In human tissues, H_4 receptor expression was most abundant in bone marrow, peripheral blood, spleen, thymus, small intestine, colon, heart and lung (Nakamura *et al.*, 2000; Liu *et al.*, 2001a; Morse *et al.*, 2001; Nguyen *et al.*, 2001; Zhu *et al.*, 2001). Discrepancies have occurred in both human and rodent tissue based on these mRNA studies, which highlights the necessity for selective immunological anti-H_4 receptor probes.

Anti-H_4 receptor antibodies have been crucial tools to study the H_4 receptor (van Rijn *et al.*, 2006; 2008; Dijkstra *et al.*, 2007; Baumer *et al.*, 2008; Dijkstra *et al.*, 2008; Morini *et al.*, 2008). The majority of cells expressing H_4 receptors are indeed hematopoietic in lineage, including neutrophils, mast cells, eosinophils, basophils, dendritic cells, monocytes and T cells (e.g. van Rijn *et al.*, 2006; Dijkstra *et al.*, 2007; Baumer *et al.*, 2008; Dijkstra *et al.*, 2008), but importantly, it has recently been shown that the H_4 receptor is not exclusively expressed on hematopoietic cells. The H_4 receptor has been identified in subsets of endocrine cells in the gastrointestinal tract (distinct from H_3R containing cells) (Morini *et al.*, 2008) and has been shown also to be expressed on dermal fibroblasts (Ikawa *et al.*, 2008). Immunohistochemical studies have shown that H_4 receptors are present on nerves from the human nasal mucosa (Nakaya *et al.*, 2004) and in the enteric nervous system on the myenteric plexus of the rodent fundus (H_3R absent) (PL Chazot, D Grandi, FC Shenton, G Morini, unpublished). Indeed, importantly, as with the other histamine receptor subtypes, the H_4 receptor is expressed in the central nervous system (Connelly, *et al.*, 2009). In agreement, the H_4 receptor was detected on subpopulations of small and medium diameter cells within the lumbar sensory dorsal root ganglia and laminae I/II of the dorsal horn of the lumbar spinal cord (Strakhova *et al.*, 2009; PL Chazot, NL Lethbridge, unpublished). These latter results indicate that the H_4 receptor is present on synaptic terminals of primary sensory afferent neurons. Therefore, the H_4 receptor is likely to subserve distinct roles in many parts of the body, and therefore may represent a target for an expanded array of therapeutic arenas, including neuropathic pain.

H_4 receptor biochemistry

Immunological approaches have demonstrated that the H_4 receptor comprises robust dimeric structures (van Rijn *et al.*, 2006; 2008). It is clear from immunoblots that higher molecular weight species (consistent with dimers and/or higher oligomers) are present in both recombinant and native tissue (Figure 4). Immunoblotting identified three major protein species in human spleen lysate. Interestingly a low molecular weight species was clearly visible in the spleen lysate at 31 kDa, likely to be the receptor monomer. In contrast, monomeric species were not evident in either lymphocytes (polyhaemagglutinnin blasts) or in brain membranes (van Rijn *et al.*, 2006; Connelly *et al.*, 2009). The observed sizes detected in immunoblotting studies are not always completely consistent with H_4R dimeric structures, with sizes ranging between 60 and 80 kD in HEK-293 H_4R-transfected cells or in other native tissue preparations (Figure 4, van Rijn *et al.*, 2006; Connelly *et al.*, 2009). Several possibilities could account for these observations, including tissue-specific complements of H_4R isoforms and differential tissue-specific post-translational modifications (e.g. palmitoylation, glycosylation).

Anti-H_4 receptor antibodies were also used to investigate the expression and potential role of the newly described

human H_4 receptor isoforms: $H_4(302)$ and $H_4(67)$ (van Rijn *et al.*, 2008). Because mRNAs coding for all three isoforms are found in several white blood cell types, hetero-oligomerization may occur in native tissue. The two shorter isoforms are both non-functional as regards ligand binding and signalling. However they may play a regulatory role as they appear to reduce number of histamine binding to the $H_4(390)$ receptor by 55% [$H_4(302)$] and 30%]$H_4(67)$] in heterologous expression studies. Based on surface biotinylation experiments, the three were expressed at the cell surface, although the shorter versions with greatly reduced efficiency. Furthermore co-expression of the $H_4(390)$ with either $H_4(302)$ or $H_4(67)$ dose-dependently reduced surface expression of the full length receptor (van Rijn *et al.*, 2008). These results provide further evidence to support the role of splice isoforms as dominant negative regulatory elements, which maybe a common theme in GPCR-regulatory pathways (Bakker *et al.*, 2006).

The majority of secreted and cell surface proteins that transit to the cell surface through the endoplasmic reticulum are N-glycosylated (95% of GPCRs) (Lanctot *et al.*, 2005). In order for a GPCR to elicit intracellular signalling appropriately, the correct quantity of properly folded functional receptors must be available in the plasma membrane. It is equally essential that signalling can be terminated at the appropriate time and the receptors either recycled or permanently degraded. The regulatory mechanisms controlling export trafficking of GPCRs, including glycosylation, have recently been reviewed (Duvernay *et al.*, 2005). The role of post-translational changes to receptors is complex and an area where there is growing interest. Several studies have shown that glycosylation is involved in GPCR transport from the endoplasmic reticulum through the Golgi to the cell surface (reviewed in Duvernay *et al.*, 2005). Both the H_3 and H_4 receptors are indeed N-glycosylated (van Rijn *et al.*, 2006; Shenton and Chazot, 2006). Interestingly, receptor dimerization *in vitro* does not appear to be dependent on N-glycosylation. However, in the case of endogenous H_4 receptor expressed in human polyhaemagglutinnin blasts, chemical deglycosylation appears to destabilize the preformed dimeric species to individual monomers (van Rijn *et al.*, 2006). The pharmacological influence of N-glycosylation of the H_4 receptor is yet to be explored.

Pharmacological tools to study the H_4 receptor

Already with the early description of histamine responsiveness of eosinophils in 1994, a clearly different pharmacology was described in comparison with the known receptor subtypes (Raible *et al.*, 1994). The subsequent cloning of the H_4 receptor cDNA and the analysis of the expressed protein also resulted in the rapid recognition of a completely distinct H_4 receptor pharmacology (Hough, 2001). Since then, major progress has been made in the discovery of selective H_4 receptor agonists and antagonists.

The early pharmacological and (patho)physiological characterization of the H_4 receptor was performed using agonists that were originally developed for the homologous H_3R receptor. Typically, these initial H_3 and H_4 receptor dual activity reference compounds all contain an imidazole heterocycle. The most important examples are the H_3 and H_4 receptor agonist, immepip (2) and the H_3 antagonist and H_4 agonist clobenpropit (3) (Lim *et al.*, 2005) (Figure 5).

The first ligand that was optimized for selective H_4 receptor activation is OUP-16 (4) (Hashimoto *et al.*, 2003) (Figure 5). This full agonist has a 40-fold selectivity for the H_4 receptor over the H_3 receptor. More recently, the potent H_4 receptor agonist 4-methylhistamine (5) was described after evaluation of a large number of histaminergic compounds (Lim *et al.*, 2005). Although originally developed in the H_2 receptor research programme, 4-methylhistamine has a high affinity for the H_4 receptor, while displaying at least a 100-fold selectivity over the other histamine receptor subtypes, including the H_2 receptor. The first identified non-imidazole H_4 receptor agonist was clozapine (6) (Oda *et al.*, 2000). This antipsychotic agent has affinity for a large number of GPCRs, but almost exclusively acts as antagonist. The dibenzodiazepine

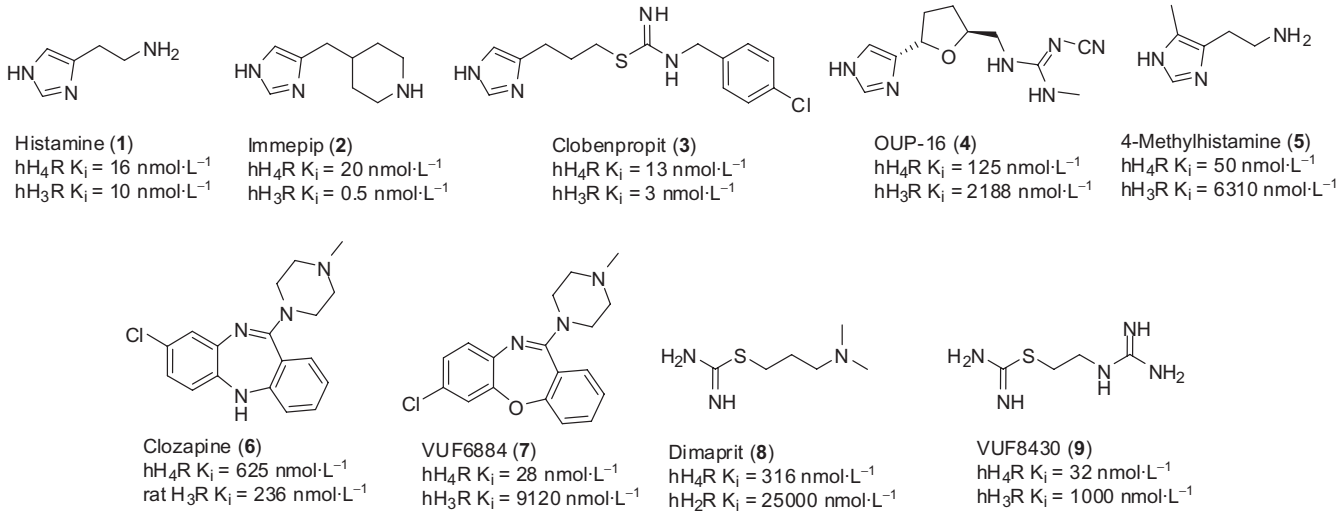

Figure 5 First and second generation H_4 receptor agonists with different selectivity profiles.

structure was optimized for H_4 receptor affinity, leading to the dual activity H_1 receptor antagonist and H_4 receptor agonist VUF6884 (7) (Smits *et al.*, 2006). Although promiscuous GPCR binders, this class of ligands provides detailed insight of the H_4 receptor agonist binding site (Smits *et al.*, 2006; Jongejan *et al.*, 2008). Another non-imidazole H_4 receptor agonist is dimaprit (8) and its analog VUF8430 (9) (Lim *et al.*, 2005; Lim *et al.*, 2006). The latter is a full agonist with high affinity for the H_4 receptor and a 30-fold selectivity over the H_3 receptor. In combination with 4-methylhistamine, VUF8430 forms a new useful pair of tools to study H_4 receptor pharmacology (see Lim *et al.*, 2009).

As is true for the first H_4 receptor agonists, the first antagonists that were used to characterize the H_4 receptor were imidazole-containing, dual activity H_3 and H_4 receptor ligands. Of particular use has been the H_3 and H_4 receptor inverse agonist thioperamide (10) (Figure 6; Buckland *et al.*, 2003; Hofstra *et al.*, 2003; Bell *et al.*, 2004; Takeshita *et al.*, 2004; Damaj *et al.*, 2007). The H_4 receptor research field has been greatly helped by the early discovery and disclosure of the selective non-imidazole neutral antagonist JNJ7777120 (11). The compound resulted from a high-throughput screening campaign and subsequent hit optimization efforts at Johnson and Johnson Pharmaceuticals (Carruthers *et al.*, 2002). JNJ7777120 is equipotently active at human, mouse and rat H_4Rs (Jablonowski *et al.*, 2003; Thurmond *et al.*, 2004). This H_4R reference antagonist is more than a thousand fold selective over other histamine receptor subtypes and was reported to have no cross-reactivity in a panel of 50 other receptors, enzymes, transporters and ion-channels. JNJ7777120 has reasonable oral bioavailability (22%), but its use in disease models is hampered by a short *in vivo* half-life of about 0.8 h (Venable *et al.*, 2005). Efforts to obtain compounds with improved properties by scaffold hopping led to VUF6002 (12) (Figure 6; Terzioglu *et al.*, 2004; Venable *et al.*, 2005). Although this benzimidazole derivative has an improved liver microsomes stability, the half-life remains limited ($t_{1/2}$ = 1.0 h).

Another series of H_4R compounds that was recently claimed in a patent by Johnson and Johnson contain methylpiperazine substituted 2-quinoxalinones (Edwards and Venable, 2005), including compound 13. At VU University Amsterdam, the construction of a H_4R pharmacophore model and rational design approaches resulted in the potent quinazoline compound 14 (Leurs *et al.*, submitted). This compound was shown to have anti-inflammatory properties *in vivo* in the rat.

Bayer Healthcare AG has recently patented aminopyrimidines (e.g. 15) as H_4 receptor antagonists (Sato *et al.*, 2005a,b). Since this disclosure, several other companies have claimed other pyrimidines as H_4 receptor ligands, including Abbott Laboratories who developed the rotationally restricted analogue A-943931 (16). This compound has been thoroughly characterized and displays a more than 640-fold selectivity over the H_3 receptor, a 190-fold selectivity over α_1 adrenergic receptors and a 470-fold selectivity over 5-HT_{1d} receptors. A-943931 is reported to have excellent antagonistic activity both *in vitro* and *in vivo* across multiple species, while displaying excellent metabolic stability and good oral bioavailability (90%). This compound is a good anti-inflammatory agent in mice and also displays good efficacy in rat pain models (Cowart *et al.*, 2008). Further exploration of the aminopyrimidine theme resulted in the recently described H_4 receptor antagonist A-987306 (17), a potent and selective compound with an excellent pharmacokinetic profile, including a half-life of 3.7 h after p.o. dosing (Liu *et al.*, 2008). A-987306 (17) has anti-inflammatory activity in a peritonitis model and is especially potent in a pain assay in rats, as was shown by the blockage of carrageenan induced thermal hyperalgesia.

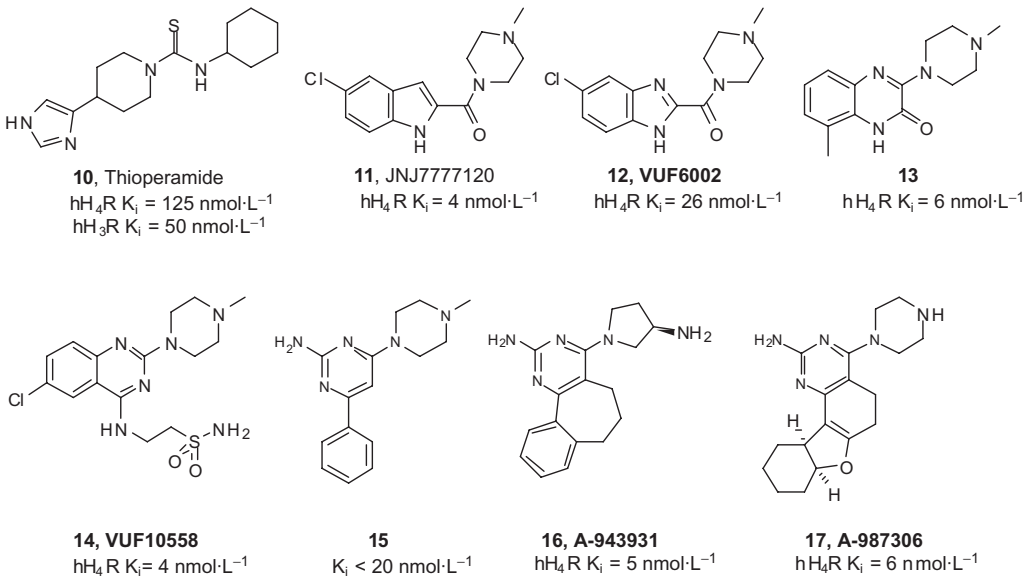

Figure 6 First and second generation H_4 receptor antagonists. Except for thioperamide (10) the antagonists display at least a 100-fold selectivity for the histamine H_4 receptor over the histamine H_3 receptor.

In order to study the pharmacology of the H_4 receptor, radioligand binding studies are instrumental. As with the H_3 receptor, the H_4 receptor binds the endogenous agonist histamine with high, nanomolar affinity (Nakamura *et al*., 2000; Oda *et al*., 2000; Liu *et al*., 2001a; Morse *et al*., 2001; Nguyen *et al*., 2001; Zhu *et al*., 2001). Consequently, [^{3}H]histamine has been effectively used as radioligand since the discovery of the H_4 receptor (Nakamura *et al*., 2000; Oda *et al*., 2000; Liu *et al*., 2001a; Morse *et al*., 2001; Nguyen *et al*., 2001; Zhu *et al*., 2001). In some studies also [^{3}H]N^{α}-methylhistamine has been used as radiotracer (Liu *et al*., 2001a), but this has not become very popular. In 2004 J&J described the use of [^{3}H]JNJ7777120 as antagonist radioligand for the H_4 receptor (Thurmond *et al*., 2004). This new radioligand binds differently than histamine in the H_4R ligand binding pocket (Jongejan *et al*., 2008), allowing the analysis of, for example, distinct H_4 receptor mutants that have lost high affinity [^{3}H]histamine binding (Jongejan *et al*., 2008).

Final remarks

With the cloning of the gene of the histamine H_4 receptor in 1999/2000 the field of histamine research has been fueled with new excitement. Several laboratories have already identified a set of useful tools (agonists, antagonists, anti-H_4 receptor antibodies), allowing the field to address the function of the H_4 receptor in both the periphery and the brain. Moreover, in the last few years a large increase in knowledge on the biochemical pharmacology of the H_4 receptor has been obtained. In view of the first preclinical *in vivo* data for H_4 receptor ligands, this new histamine receptor family member is expected to become an important target in the area of inflammatory disorders and neuropathic pain. Moreover, also pruritis is associated with H_4 receptor function as itching can be induced by H_4 agonists such as clobenpropit and 4-methylhistamine and blocked by H_4 antagonists such as JNJ7777120. It might nevertheless be an interesting challenge to develop H_4 receptor drugs, as so far relatively high levels of H_4 receptor antagonists are required to inhibit *in vivo* inflammatory effects despite the high potency and selectivity of the tool compounds. This may reflect limitations of the tool compounds with respect to for example pharmacokinetic properties, as well as for example high local histamine concentrations at the H_4 receptor.

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Conflict of interest

None.

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