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Molecular pharmacology of histamine H4 receptors

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1. ABSTRACT

The histamine H4 receptor (H_4R) is the youngest member of the histamine receptor family. Based on its predominant expression pattern in hematopoietic cells, the H_4R is considered to be an interesting drug target for inflammatory disorders such as allergy and asthma. Since the identification and cloning of the H_4R in 2000, drug discovery programs boosted the development of various H_4R (specific) ligands. Differences between H_4R orthologs in combination with available three-dimensional G protein-coupled receptor (GPCR) models have guided site-directed mutagenesis studies to gain insight in ligand binding and receptor activation. In addition, ongoing characterization of H_4R-mediated signaling in transfected and native cells contributes to further unravel the (patho-) physiological functions of H_4Rs.

2. INTRODUCTION / HISTORY

The small biogenic amine histamine was identified as an important mediator in a variety of physiological processes in the beginning of the 20th century (1-3). Histamine is produced de novo by L-histidinedecarboxylase (HDC)-catalyzed decarboxylation of the amino acid L-histidine, and is stored in cytoplasmic granules in immune cells (e.g. eosinophils and mast cells), enterochromaffin-like cells in the stomach and in neurons. Contact of eosinophils or mast cells with an allergen results in immunoglobulin cross-linking and release of stored histamine from the granules (4). Histamine causes the well-known allergic responses, such as sneezing, coughing, rhinorrea and vasoconstriction. Drugs inhibiting these allergic symptoms were initially classified as

antihistamines and are now known to act through a receptor protein called histamine H1 receptor (H_1R).

In the stomach, histamine stimulation increases gastric acid secretion. The different pharmacology that was found for some histaminergic ligands in airway smooth muscle, heart, uterus and stomach, indicated that histamine affects at least two distinct receptors, namely the H_1R and histamine H2 receptor (H_2R) (5). Unambiguous evidence for the existence of two different receptors arose from the development of the first (H_2R-) selective antagonists burimamide and metiamide by Sir James Black and colleagues (6). These compounds prevented the histamine-induced release of HCl in the stomach, but did not affect the responses supposed to be mediated by the H_1R. Consequently, in the following years a plethora of selective drugs was developed against either H_1R or H_2R to relief either allergic symptoms or inhibit abundant gastric acid release in the stomach, respectively. These H_1R (antihistamines) and H_2R antagonists became worldwide blockbuster drugs that are now readily available from the local drugstore without a prescription. The sedative effects of the first generation antihistamines revealed the presence of H_1R receptors in the brain. To prevent drowsiness as side effect, a second generation of H_1R antihistamines was developed, that could not pass the blood brain barrier. At the same time, brain-penetrating antihistamines were marketed and even newly developed as sleep aid.

In the eighties, a new histamine receptor that could not be inhibited according to the known pharmacology of the H_1R or H_2R antagonists was identified in rat brain (7). This new receptor was classified as the histamine H3 receptor (H_3R) and is now known to be involved in the regulation of e.g. sleep/wake cycle and cognitive processes, by presynaptically inhibiting the release of histamine, but also other neurotransmitters like dopamine, noradrenaline, acetylcholine and serotonin. The H_3R is seen as a potential target in central nervous system (CNS) disorders such as Alzheimer's disease, schizophrenia and attention deficit hyperactivity disorder (ADHD) (8, 9).

In addition to the "classical" techniques such as *ex vivo* organ-based studies to pharmacologically define histamine-induced effects, new molecular biology techniques became available in the late twentieth century, eventually leading to the sequencing of complete genomes. Thanks to these developments, histamine receptors can now be recombinantly expressed in convenient host cell lines and biochemically and pharmacologically characterized. Moreover, using site-directed mutagenesis the role of specific amino acids in protein function can be studied. In 1991, both H_1R- and H_2R-encoding genes were readily cloned, but it lasted until 1999 before the gene of the H_3R was identified (10). The histamine receptor proteins are all classified as G protein-coupled receptors (GPCRs), which are characterized by seven transmembrane (TM) alpha-helices. In a quest for new GPCRs that show homology to the H_3R gene, a deduced sequence was found that shares considerable amino acid similarity. Several groups cloned this new gene at the same time, and the corresponding protein was named histamine H4 receptor (H_4R) (11-16).

In this essay we will give a broad overview of past en present H_4R research, as well as highlight some of the emerging developments in the H_4R field.

3. H_4R GENE

The protein encoded by the human histamine H_4R gene (i.e. HRH4) shows very low (~19%) amino acid sequence homology to the histamine H1 and H2 receptors and shares ~37% identity with the H_3R. The HRH4 is 16.98kb in size and is mapped by radiation hybrid experiments on chromosome 18q11.2 as a single copy (17). Increased copy numbers of HRH4 are associated with the occurrence of the autoimmune disorder systemic lupus erythematosis (SLE), arthritis, and proteinuria, due to elevated H_4R expression levels (18). On the other hand, lower copy numbers are associated with decreased proteinuria (18).

Transcription of HRH4 results in a mRNA molecule of 3.7kb. The open reading frame is 1173bp in size and encodes 390 amino acids. The HRH4 contains two introns of 7867 bp and >17500 bp, dividing the actual coding region into three exons (amino acids 1-65, 66-119 and 120-390) (Figure. 1). The HRH3 and HRH4 share a similar intron/exon distribution (19), suggesting that they have evolved from a common ancestral gene. In contrast, HRH1 and HRH2 are intron-less in their coding region (20, 21). Four alternative splicing variants of HRH4 have been identified so far, namely H_4R (302), H_4R (67) (Figure. 2) and H_4bR and H_4cR. The H_4R (302) splice variant contains TM1, part of TM2 and TM5-7, whereas H_4R (67) is truncated due to a frame shift and only contains TM1 and the first half of TM2 (22). Both these HRH4 splice variants are predominantly located intracellularly and are not able to activate signaling pathways. However, these splice variants act as dominant negative partners for the full length H_4R (see 5.1). The splice variants H_4bR and H_4cR were cloned from human spleen cDNA by Merck (patent WO 03/020907 A2). The H_4bR is identically spliced as H_4R (67), whereas H_4cR contains exon 2 and recognizes an alternative acceptor site in exon 3, which causes a 33 amino acid deletion. In contrast to H_4R (302) and H_4R (67), both splice variants are reported in a patent to bind [^{3}H]-histamine and to activate downstream pathways like cAMP inhibition, Ca2+ release and MAPK activation (22), but this has so far not been confirmed.

The promoter region of HRH4 contains several binding motifs for transcription factors (TF), such as nuclear factor kappa B, nuclear factor to interleukin 6, interferon regulatory factor and interferon-stimulated response element (17), but lacks TATA or CAAT box sequences. This suggests that HRH4 expression could be induced by inflammatory factors like interferon, TNFa or IL-6.

Numerous single nucleotide polymorphisms (SNPs) have been identified in the HRH4. Most SNPs are located in non-coding regions with 37 SNPs in intron 1, 45 in intron 2 and 21 in the 3' untranslated region. However, four SNPs were identified in the coding region, resulting in

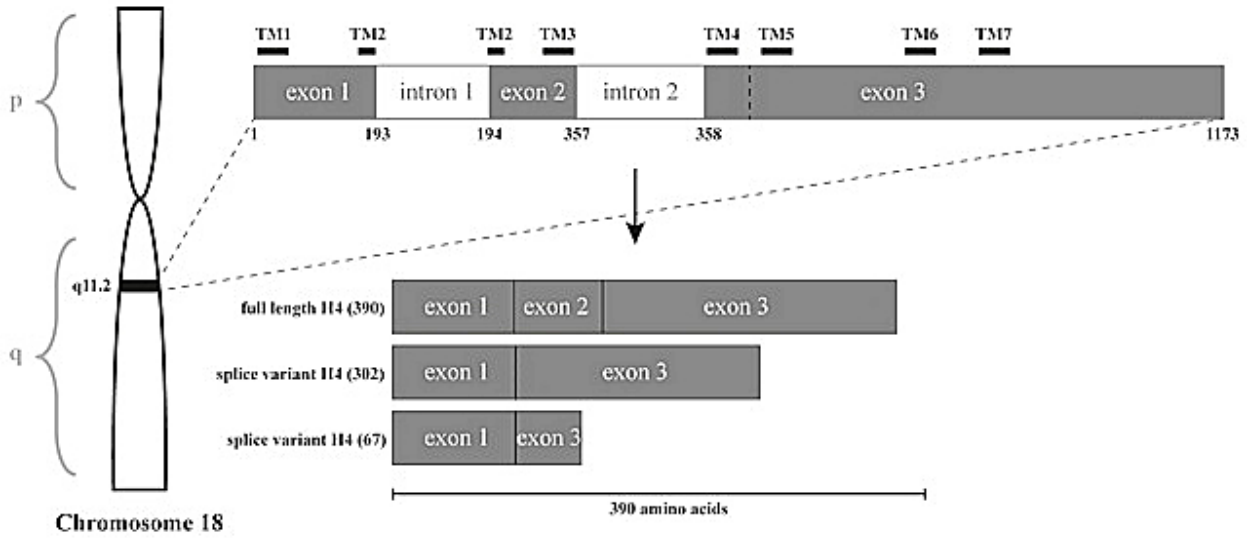

Figure 1. Schematic representation of the H_4R gene location on chromosome 18 and an overview of the exon/intron distribution the different splice variants. The gene is located on position q11.2 and consists of three exons and two introns. The regions that encode the typical 7 TM domains of the full length H_4R (390) are shown above the sequence. The splice variant H_4R (302) does not contain exon-2 and recognizes an alternative acceptor site (dotted vertical line) in between the TM4 and TM5 coding sequence. The H_4R (67) variant passes over exon-2, continues at the normal acceptor site of exon-3, which subsequently results in a frame shift and premature stop codon.

$Val^{4.48}Ala$, $Arg^{5.70}His$, $Cys^{6.16}Ser$ or a frame shift at $Leu^{7.75}$. The first two changes give rise to the differences between the HRH4 sequences as published by Oda *et al* (12) and other research groups. Recently, three SNPs (i.e. ss142022671, ss142022677 and ss142022679) were associated with atopic dermatitis (23). The SNP ss142022671 is located inside a consensus transcription factor binding motif in intron 1, resulting in increased transcription. On the other hand, the SNPs ss142022677 and ss142022679 are located in exon 3, resulting in the substitution of the $Lys^{7.71}$-encoding sequence by a stopcodon or mutation of $Lys^{7.71}$ to Ile, respectively. Moreover, when both these SNPs are present $Lys^{7.71}$ is changed to Leu (23). Unfortunately, the effects of these SNPs on H_4R pharmacology are not yet resolved.

Soon after the identification of the human HRH4, corresponding orthologs were cloned from mouse, rat, guinea pig, pig, monkey and dog (24, 25). The amino acid similarity between monkey and human H_4R is the highest with 93%, but decreases when comparing with rodent or pig H_4R proteins (65-72%, respectively) (Figure. 2) (24, 26-28). Unraveling sequence differences between species is an interesting study for evolutionary scientists. However, it has proven to be a very useful tool to delineate ligand-binding sites as well (see 7.1).

4. H_4R EXPRESSION PROFILE

The HRH4 is expressed in a variety of tissues such as bone marrow, spleen, peripheral blood, thymus, small intestine, colon, heart and lung, as revealed by quantitative polymerase chain reactions (qPCR), Northern blot analysis, microarray analysis or *in situ* hybridization (29-33). However, HRH4 seems to be predominantly expressed on cells of hematopoietic origin, e.g. eosinophils, mast cells, basophils, neutrophils, dendritic cells, monocytes and T cells (Figure. 3). HRH4 mRNA of the splice variants could be detected in pre-monocytes and eosinophils (22). In addition, HRH4 expression was also detected in subsets of endocrine cells in the gastrointestinal tract (34), dermal fibroblasts and in the central nervous system (35). There is evidence of HRH4 expression in the mouse brain (14), but human data is still contradictory (13, 15, 17, 24, 36). Although the above-mentioned techniques detect HRH4 expression, they obviously do not give an indication regarding H_4R protein levels.

A useful tool to detect H_4R proteins on cells and tissues is a H_4R specific antibody such as the polyclonal anti-H_4R antibody developed against the last 17 amino acids of the C-tail (32). H4R proteins have been detected by antibody-based immunofluorescent staining in human monocyte-derived dendritic cells (MoDC) (29) as well as on primary Langerhans cells from murine and human skin samples (37). Moreover, it was observed that H4Rs are upregulated during the differentiation from monocytes into MoDC (38). More recently, this anti-hH_4R receptor antibody was used to localize human and mouse H_4R protein in the CNS (35). Histamine H_4Rs are expressed in distinct deep laminae (particularly layer VI) in the human cortex and mouse thalamus, hippocampal CA4 stratum lucidum and layer IV of the cerebral cortex. In contrast, very low H_4R expression was observed in the striatum and the remaining subfields of the hippocampus (35). The antibody data were confirmed by H_4R-mediated electrophysiology responses in layer VI somatosensory cortex neurons in mice (35). In addition, H_4R protein was

Figure 2. Phylogenetic tree of the human histamine receptors and H_4R orthologs. Sequences are downloaded from NCBI website, and the graph was created with ClustalW. Alignment parameters from the Gonnet series were used.

also detected on mouse spinal cords motor neurons (39). On nerves of human nasal mucosa, H4R proteins were found to colocalize with both H1R and H3R (40). H1R, H2R, and H4R proteins are expressed in human intestinal tissue (41). Interestingly, both H1R and H4R expression was significantly decreased in colorectal tumors (41).

5. H_4R PROTEIN STRUCTURE AND POST-TRANSLATIONAL MODIFICATIONS

Histamine receptors belong to the family of GPCRs. These receptor proteins consist of seven cell membrane-spanning alpha-helices that are connected by three extracellular loops (ELs) and three intracellular loops

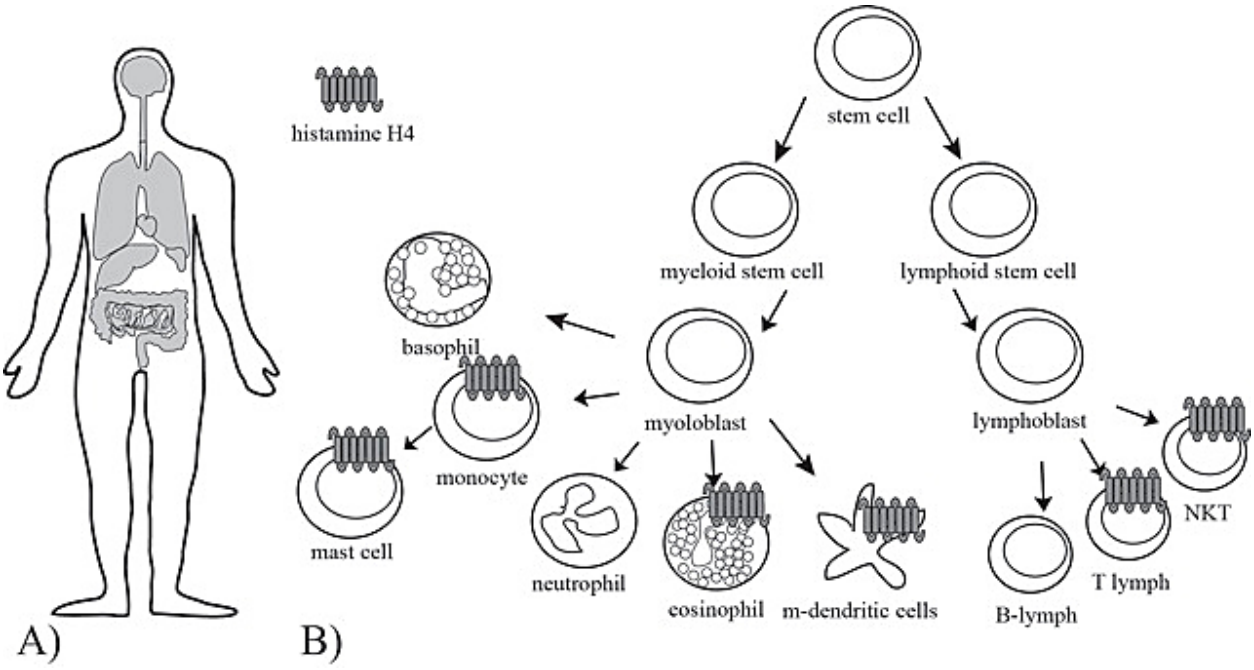

Figure 3. H_4R distribution in the human body. A) Organs and tissues that express H_4R are drawn and colored gray. B) H_4Rs are predominantly expressed in hematopoietic cells. Data were either obtained from mRNA detection via micro-array and qPCR or protein detection via H_4R specific antibodies.

(ILs). The N-terminal tail is located extracellular and the C-tail intracellular (Figure. 4). The histamine receptors have several conserved structural motifs that are common to the class A (rhodopsin-like) GPCRs, including the highly conserved residues $Asn^{1.50}$, $Asp^{2.50}$, $Arg^{3.50}$, $Trp^{4.50}$, $Pro^{5.50}$, $Pro^{6.50}$ and $Pro^{7.50}$ (43). Like in most other GPCRs, (42) TM3 and the EL2 of H_4R are presumably connected by a disulphide bridge between $Cys^{3.25}$ and $Cys^{45.50}$. The extracellular N-terminal tail contains two asparagines ($Asn^{1.21}$ and $Asn^{1.25}$) that are predicted to be involved in post-translational glycosylation, whereas the intracellular C-tail is possibly anchored to the cell membrane through palmitoylation of the $Cys^{7.69}$ residue (Figure. 4). In addition, like most GPCRs, H_4R has an additional helix 8 that is located intracellularly and does not span the cell membrane.

5.1. H_4R assembly in the cell membrane

In the era of the first two histamine receptors it was generally believed that GPCRs function as monomeric entities. However the last two decades, a substantial amount of literature was published showing the existence of higher order structures consisting of two or more GPCRs (Figure. 5). One of the most striking examples was given by two class C GPCRs, the $GABA_{B1}$ and $GABA_{B2}$ receptors. The $GABA_B$ proteins are obligatory heterodimers, not able to reach the cell surface or signal in the absence of one another (44-47). Although there is increasing evidence of GPCRs interacting with each other, the functional consequences of class A GPCR di- or oligomerization are less clear. Hence the quaternary organization for this class of GPCRs is not indisputably accepted (48).

All four histamine receptors form higher order assemblies (49-52). H_4R dimers were detected in native cells (e.g. human phytohaemagglutinin (PHA) blasts and spleen lysates) using a selective anti-H_4R antibody in co-immunoprecipitation experiments (32). However, co-immunoprecipitation experiments are disputable because of a-specific aggregation after disruption of the cellular environment. Therefore, supporting evidence for dimerization was obtained in transfected living cells (e.g. HEK293 and COS7 cells) that express H_4R proteins at physiological relevant (~300 fmol/mg membrane protein) (32). Both homo- and heteromeric complexes were identified for the H_4R using biophysical (bioluminescence & time-resolved fluorescence resonance energy transfer) techniques (32, 53). Moreover, H_4R dimers are localized at the cell surface, as demonstrated by antibody-based time-resolved fluorescence resonance energy transfer (trFRET) measurement, that only allows detection of cell surface complexes.

H_4R oligomers are formed constitutively and their formation is not modulated by (inverse) agonists or antagonists. H_4R oligomerization does not require posttranslational N-glycosylation, although a possible role for glycosylation in stabilization of the complex was suggested, based on a decrease in the dimeric population upon deglycosylation (32). H_1R-H_4R heteromeric complexes are only detected at very high expression levels, and are most likely the consequence of random interactions.

The HRH4 splice variants H_4R (67) and H_4R (302) form complexes with the full length H_4R, and retain

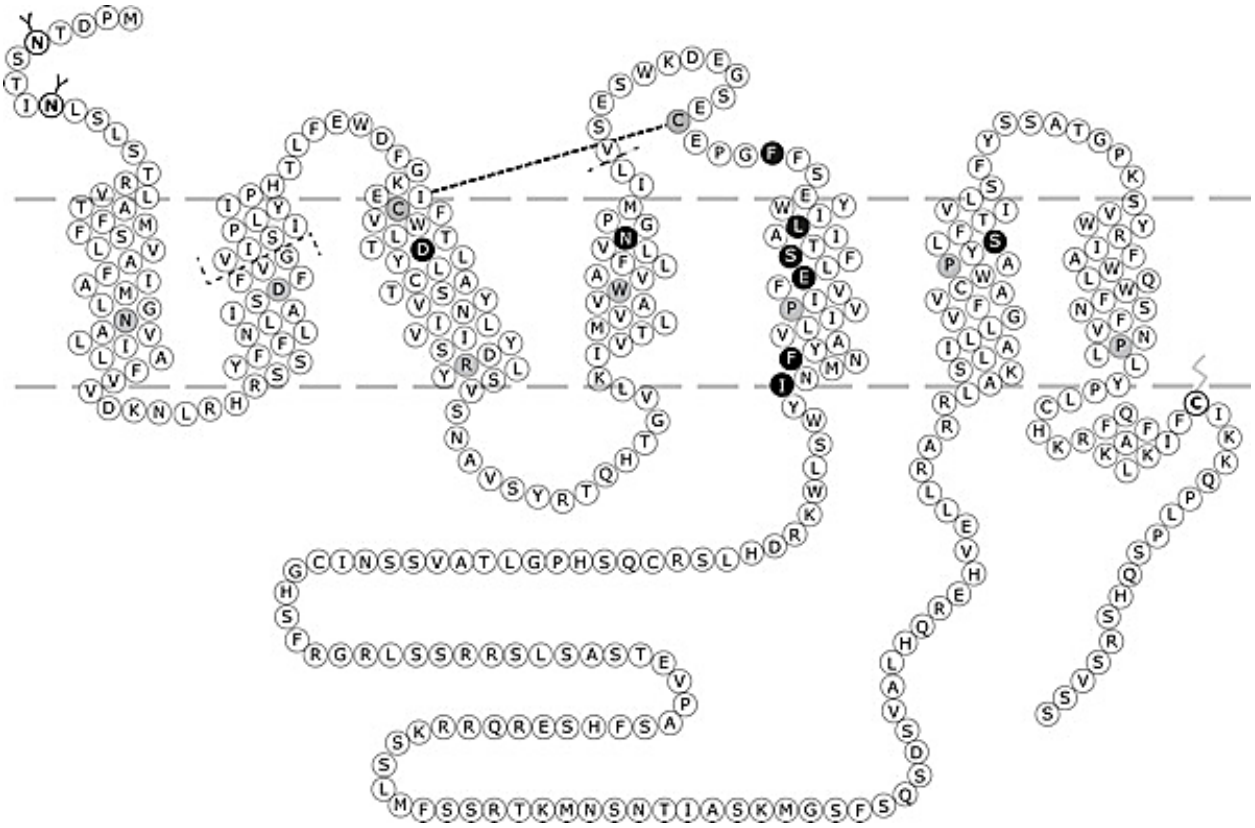

Figure 4. Snake plot of the human H_4R protein. The cell membrane-associated H_4R has 7TM helices and several conserved structural motifs that are common to class A (rhodopsin-like) GPCRs. A disulphide bridge between the conserved $Cys^{3.25}$ and $Cys^{45.50}$ (gray circles) connects the first EL/TM3 and the second EL. The N-terminal extracellular tail contains two asparagines ($Asn^{1.21}$ and $Asn^{1.25}$) (bold) that are possibly involved in post-translational glycosylation. The intracellular C-tail is presumably anchored to the cell membrane through palmitoylation of the $Cys^{7.69}$ residue (bold). Residues that have been shown to be important in ligand binding and/or receptor activation as well to be responsible for observed differences in ligand binding between species are shown in black circles with white text.

the latter intracellularly in a dominant negative manner (22). Hypothetically, since the mRNA of these splice variants is differentially expressed in different cell types, it could well be that they have a role in the regulation of H_4R expression at the cell surface (22).

6. H_4R LIGANDS

Since the discovery of the H_4R, numerous ligands have been identified that bind the receptor and affect downstream signaling pathways. Several efforts have been made to design and synthesize H_4R selective (inverse) agonists and antagonists. Considering the amino acid similarity to the H_3R, especially in the ligand binding pocket that is formed by the TM domain, it is not surprising that the majority of imidazol-containing H_3R ligands have affinity for the H_4R as well (54) (Figure 6). Examples include R-a-methylhistamine (RAMH), immepip (both 40-fold selective for H_3R), immetridine and methimepip (300 and 2000-fold H_3R selective, respectively). Small changes in ligand structure result in great differences in histamine receptor subtype specificity. Even so remarkable is the change in efficacy for some compounds on the distinct histamine receptor subtypes. The H_3R antagonist clobenpropit, for example, acts as a high affinity partial agonist at the H_4R.

Besides H_3R cross reactivity, some H_4R ligands are able to bind H_1R or H_2R (Figure. 6) (55). The majority of these shared ligands contain the characteristic imidazole heterocycle, with the exception of clozapine (analogues) (56). Interestingly, clozapine binds promiscuously to several GPCRs, but only acts as an agonist on H_4R (36).

6.1. H_4R agonists

Optimization of the dibenzodiazepine clozapine resulted in the rigid structure VUF6884, a H_1R antagonist and H_4R agonist (57). Another non-imidazole H_4R agonist is the dimaprit analog VUF8430 (54, 58). The latter is a full agonist with high affinity for the H_4R and 30-fold selectivity over the H_3R. In contrast to the H_3R, H_4R agonists are thus not limited to imidazole containing structures. Ligand optimization studies and evaluation of other histamine receptor compounds resulted in more selective H_4R agonists. OUP-16 (59) and 4-methylhistamine (54), display respectively 40-fold and

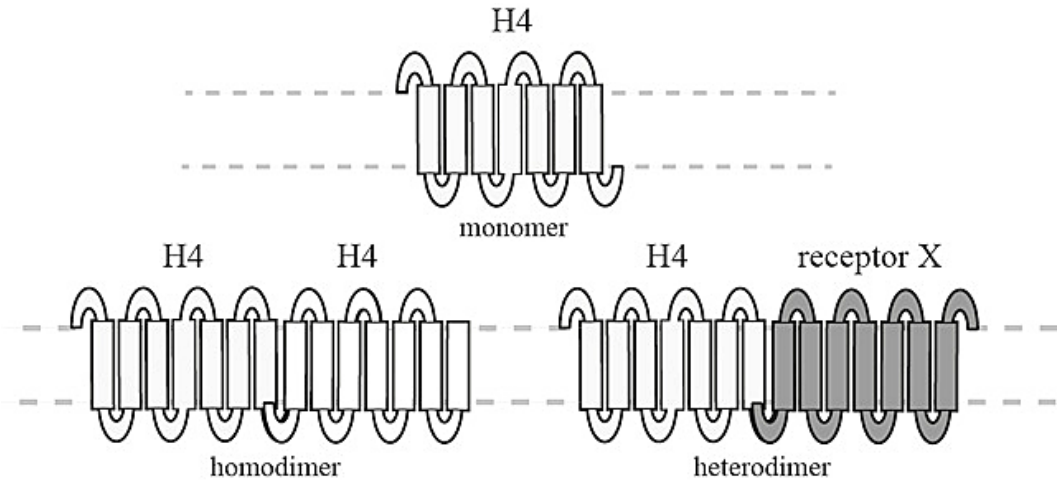

Figure 5. H_4R organization in the cell membrane. H_4Rs were shown with biochemical and biophysical methods to constitutively dimerize both in native cells and transfected cells. H_4R can form homodimers consisting of two H_4R proteins or heterodimers consisting of H_4R and another GPCR.

100-fold H_4R selectivity over the other histamine receptor subtypes.

In more recent years new classes of H_4R agonists were discovered, each with their advantages and disadvantages. The acylguanidine agonists were developed as H_3R/H_4R interacting compounds and can be useful to study H_4R pharmacology in absence of H_3R (e.g. in some immune cells). Exchange of the acyl group with a cyano group resulting in the cyanoguanidines, improves H_4R selectivity (60). UR-PI376 shows 30-fold binding selectivity over H_3R, but shows a drop in potency on mouse H_4Rs. An interesting aspect of this H_4R agonist is that it is unable to activate any other histamine receptor subtype (61).

Recently, Johnson and Johnson (J&J) published their newest class of H_4R agonists, the oxime analogues of JNJ 7777120 (see below) and JNJ 10191584 (VUF6002) (62). They show low affinity for the other histamine receptor subtypes and very promising, maintain their efficacy on H_4R species orthologs (except for dog H_4R) (62). Another interesting class of H_4R agonists is the 2-arylbenzimidazoles. They were also developed by J&J and the best compound of this series has subnanomolar affinity for H_4R. This compound is highly selective by showing more than 600-fold selectivity over the other histamine receptor subtypes, but is unfortunately less potent on the mouse H_4R. Interestingly, minor changes in this 2-arylbenzimidazoles series lead to an efficacy shift from agonist into antagonists (63).

6.2. H_4R antagonists/inverse agonists

One of the first identified antagonists (later discovered to be inverse agonist) was thioperamide, but this compound is equiactive on both H_3R and H_4R receptors (64-67). The first H_4R-selective (non-imidazole) neutral antagonist JNJ 7777120 (>1000 fold selective over other histamine receptor subtypes) was discovered by J&J following a high throughput screen. This compound has equal affinity for human, mouse and rat H_4Rs (68, 69) initially making it a valuable compound to extend the *in vitro* pharmacology to *in vivo* studies. Intriguingly, the more research is performed on this compound, the more "active" it becomes. It was reported that in a steady-state GTPase assay JNJ 7777120 acts as a partial inverse agonist on the human H_4R. In the same assay, however, it behaves as a partial agonist on mouse, rat and dog H_4Rs (70). Very recently JNJ 7777120 was identified as partial biased agonist, able to recruit beta-arrestin in a G protein-independent manner (71) (see 8.4). Hence, the usefulness of this compound as reference neutral H_4R antagonist is currently under debate. JNJ 7777120 has a poor half-life (2h) upon oral administration to rats (72, 73), which should be taken into account when using it for *in vivo* H_4R targeting.

Another class of H_4R antagonists that has gained enormous interest from industry is the aminopyrimidines. According to filed patents, Bayer Healthcare AG, Palau Pharma, Pfizer and J&J all have their current research programs based on these structures. Also Abbott Laboratories followed this series of pyrimidines, eventually leading to the anti-inflammatory A-987306 (Ki H4R = 5.8 nM) with very good pharmacokinetic properties and an *in vivo* half-life up to 3.7 hours (74).

The new class of quinoxalinone H_4R ligands was found in a fragment-based drug discovery project at the VU University Amsterdam as well as at J&J, where they originated from 5-HT3 ligand discovery projects (75). The VU University Amsterdam developed these quinoxalines by combining clozapine and JNJ 7777120 in a pharmacophore model. These compounds were shown to have anti-inflammatory properties in a carrageenan-induced paw-edema model in rats (76). Subsequent scaffold hopping resulted in the discovery of inverse agonists quinazolines (56). Interestingly those structures can be extended with a sulfonamide group (resulting in the inverse agonists quinazoline sulfonamides) with a variety of

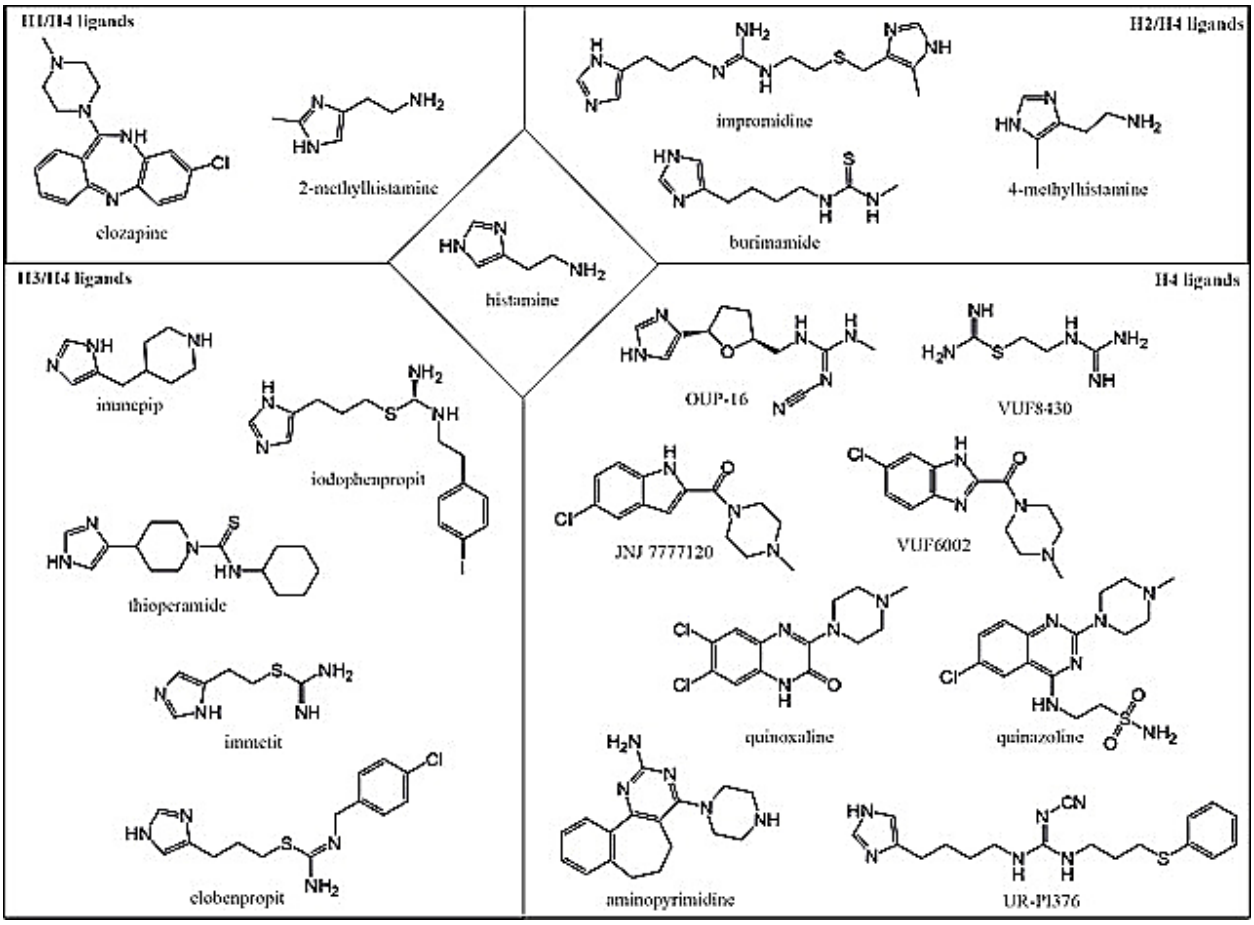

Figure 6. Chemical structures of H_4R ligands and their binding to other histamine receptor subtypes.

substituents without loosing affinity for the H_4R (77). H_4R ligands and their clinical applications have been extensively discussed in recent reviews by Smits *et al.* (78) and Engelhardt *et al.* (79).

6.3. H_4R radioligands

The agonists histamine and UR-PI294 (61) can be readily labeled with tritium and used as radioligand in ligand/receptor binding studies. Since histamine and UR-PI294 interact with high affinity to other histamine receptor subtypes (i.e. H_3Rs), only 4-methylhistamine is a H_4R-selective radioligand.

The only available H_4R antagonist radioligand is [^{3}H]-JNJ 7777120 (69), which is particularly useful when studying mutant H_4Rs that are unable to bind histamine.

7. ELUCIDATION OF LIGAND BINDING MODES IN H_4R

By relating distinct pharmacology of H_4R orthologs to their sequence divergence, information can be obtained on ligand binding modes (Figure. 7) and receptor activation. To this end, chimeras between H_4R of human and other species have been constructed and evaluated, subsequently followed by site-directed mutagenesis to pinpoint the specific amino acid (s) involved in ligand interaction.

7.1. Species differences

Recently, an extensive study was performed in which H_4Rs of several species were characterized (28). These H_4R orthologs were heterologously expressed and binding affinities of several well-known ligands were determined. Histamine and 4-methylhistamine show an equal trend when comparing their H_4R binding affinities between species. VUF8430 (58) shows a different pattern with a decreased affinity for pig, dog, and guinea pig H_4R, in comparison to other H_4R orthologs. Clozapine loses affinity for the pig, dog, mouse and rat, compared to the human H_4R. Interestingly, monkey and guinea pig show higher affinity for this tricyclic compound (28). Clobenpropit has lower affinity for pig and dog H_4R, but similar affinities for the other tested H_4R species variants, whereas the inverse agonist thioperamide has equipotent affinity for all H_4R orthologs. Both antagonists JNJ 7777120 and VUF6002 show significant lower affinity for monkey, pig, dog and guinea pig receptors as compared to the other tested species.

Most of the tested ligands show significant differences in affinity at the different species variants (24). Therefore, careful *in vitro* pharmacological characterization of H_4R orthologs is of major importance as several animal models are used to study H_4R (ligands) function *in vivo*.

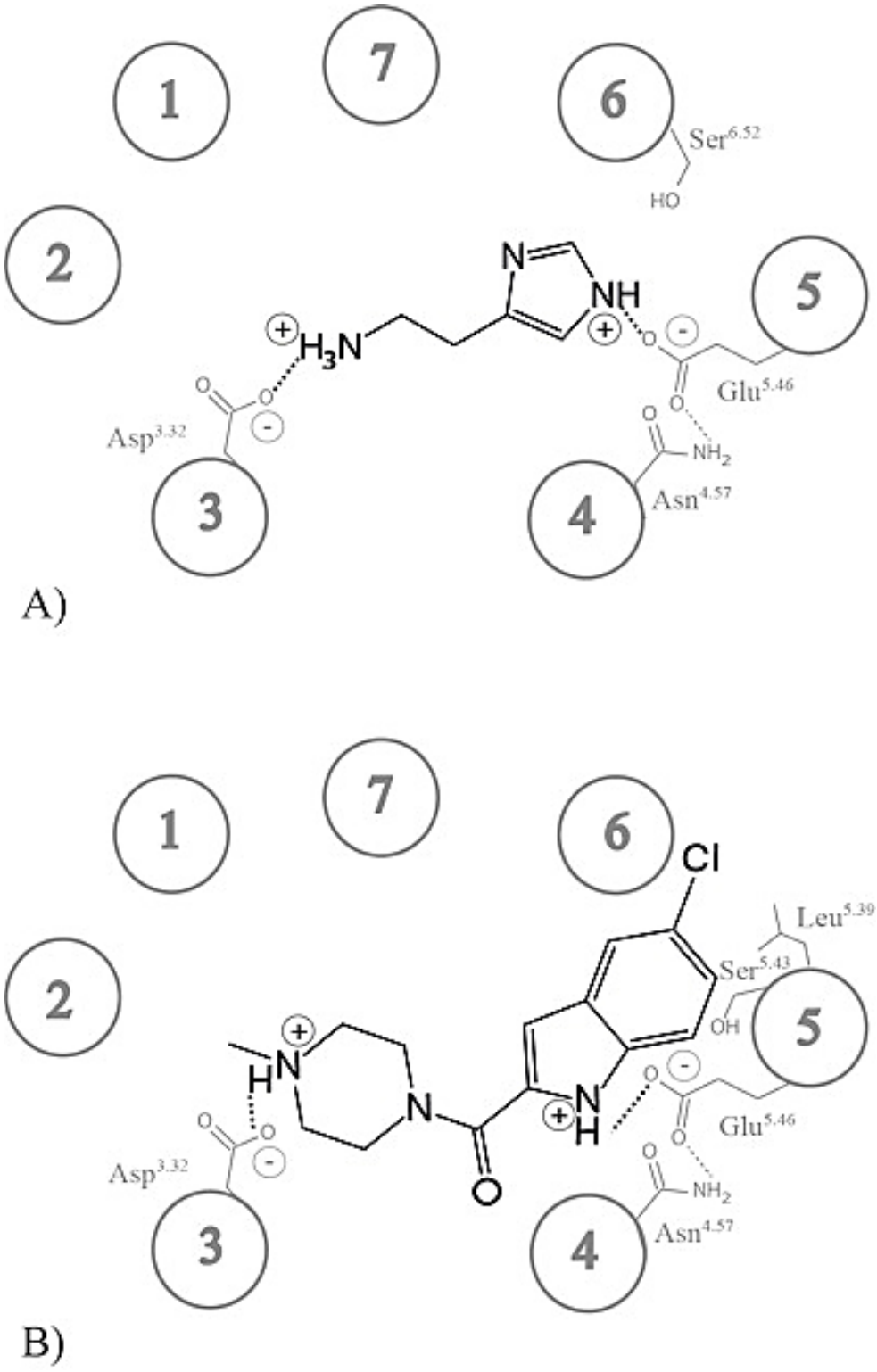

Figure 7. a) Schematic 2D representation of histamine and its interactions with H_4R residues. Based on side-directed mutagenesis (SDM) studies $D^{3.32}$ and $E^{5.46}$ are identified as the key interacting residues (83, 84). $Ser^{6.52}$ and $Asn^{4.57}$ are not crucial for binding but mutations at these positions have an effect on histamine-induced receptor activation (28). b) Schematic 2D representation of JNJ 7777120 and its interactions with H_4R residues. In SDM studies $D^{3.32}$ was identified as the major anchor point for JNJ 7777120. $E^{5.46}$ plays a less pronounced role compared to histamine binding and $L^{5.39}$ is important for the position of the chlorinated ring (28).

Considering the H_4R differences between animals, caution should be taken in selecting appropriate species to evaluate specific ligands. JNJ 7777120 is currently the most commonly used reference compound to block H_4Rs in animal models (55). However, recent developments request re-evaluation whether JNJ 7777120 should still be used as reference antagonist (see sections 6.2 and 8.4).

Chimeras between human and pig H_4Rs, followed by site-directed mutagenesis, identified residues at positions 45.55, 4.57, 5.39 and 5.43 responsible for the observed species differences in ligand affinity (28). Residue 5.39 was shown to be also responsible for the differences in ligand binding between human and monkey (28), whereas $Phe^{45.55}$ in EL2 (Figure 4) of the human H_4R was identified to be responsible for the increase in agonist affinity compared to mouse H_4R (80).

7.2. Binding pockets in H_4R

H_4R ligands bind the receptor in the common binding pocket located in the cavities formed by the TM helices. This binding pocket consists of two subpockets, which are designated i and ii (81). Pocket i is located between TM domains 2, 3 and 7, whereas pocket ii is located between TMs 3, 4, 5 and 6. Besides these general pockets, two hydrophobic subpockets exist in pocket ii. The first hydrophobic subpocket is located between TM3, TM4, TM5 and TM6 in the vicinity of $Trp^{6.48}$. The second hydrophobic subpocket is situated between TM3, TM5, TM6 and EL2 (and EL3) towards the extracellular side of the receptor (82). Compound classes such as the aminopyrimidines, quinoxalines and quinazolines are proposed to occupy these subpockets with their hydrophobic moieties (82).

7.3. Binding mode of histamine

Although the similarity with the other histamine receptor family members is relatively low (55), earlier identified key residues in ligand binding to the H_1R-H_3R, are also present in the H_4R (83). The amine group of histamine interacts with $Asp^{3.32}$ (83, 84) in subpocket i of H_4Rs and the protonated nitrogen of the basic imidazole ring is forming a hydrogen bond with $Glu^{5.46}$ in subpocket ii (Figure. 7) (83, 85). Interestingly, both in H_3R and H_4R a Glu residue is present at position 5.46, compared to Asn in H_1R and Thr in H_2R. The importance of $Glu^{5.46}$ is also shown by the inability or decrease in binding of [^{3}H]-histamine to H_4Rs in which $Glu^{5.46}$ is substituted with Ala or Gln, respectively (83, 84). It is hypothesized that the presence of this negatively charged residue is the reason for increased binding affinity of histamine to H_3R and H_4R as compared to H_1R and H_2R (83). $Asn^{4.57}$ and $Ser^{6.52}$ were also suggested to be important residues in histamine-induced activation of H_4R (84), but do not play a direct critical role in histamine binding (28). The non-imidazole small molecule agonist VUF8430 is believed to bind in a similar binding mode as histamine (83). In contrast, for the tricyclic agonist clozapine the interaction with residue $Glu^{5.46}$ is less pronounced, as illustrated by its ability to bind the mutated H_4R-$Glu^{5.46}$ (83, 86, 87).

7.4. Binding mode JNJ 7777120

Although JNJ 7777120 binds in the same binding cavity as histamine, the actual binding mode is proposed to be different. This is supported by the fact that [^{3}H]-histamine binding is almost lost in an $E^{4.56}Q$ mutant, but [^{3}H]-JNJ 7777120 is still able to interact with this mutant receptor. The positively charged piperidine nitrogen atom is believed to form a hydrogen bond with $Asp^{3.32}$ (83). The indole nitrogen atom donates a hydrogen bond to the carboxylate group of $Glu^{5.46}$. Considering the drop in affinity for JNJ 7777120 on the $L^{5.39}V$ mutant compared to a increase in affinity for clozapine for the same H_4R mutant, the chlorinated aromatic ring of JNJ 7777120 is believed to be positioned in the vicinity of this residue (Figure. 7) (86).

The binding mode of a variety of H_4R ligands has recently been extensively reviewed by Istyastono *et al.* (86).

8. SIGNALING OF H4R

Although H_4R signaling has been studied in cells endogenously expressing H_4Rs, determination of the exact mechanisms of H_4R functioning as well as ligand characterization is predominantly performed in H_4R transfected cells. In HEK293T cells, transfected with H_4R and a cAMP-response element (CRE) reporter gene construct, H_4R activation inhibits forskolin-induced cAMP production by adenylyl cyclase (AC) and subsequent CRE-driven gene transcription in a pertussis toxin (PTX)-sensitive manner, indicating the involvement of $Galpha_{i/o}$ proteins (14, 16, 36). Interestingly, no changes in cAMP levels were observed in H_4R-expressing HEK293 cells in response to histamine, even though other $Galpha_i$-mediated responses (mitogen-activated protein kinase (MAPK) phosphorylation and PTX sensitivity) could be measured (15). In addition, in the presence of chimeric $Galpha_{q/i1/2}$, $Galpha_{q/i3}$ or $Galpha_{16}$ proteins H_4R stimulation resulted in increased calcium mobilization in transfected CHO, COS-7 and HEK293 cells (15).

Furthermore, H_4R stimulation resulted in an increase in [^{35}S]-GTPgammaS binding in SK-N-MC and HEK293 cells. Interestingly, increased basal levels are observed in this assay when comparing H_4R expressing cells with control cells. Thioperamide inhibits this basal signaling. The same phenomenon is seen in Sf9 insect cells co-expressing mammalian $Galpha_{i2}$ and $Gbeta_1gamma_2$ proteins (88). This indicates that H_4Rs are constitutively active (i.e. signal in absence of a ligand) and thioperamide is acting as inverse agonist (54). Constitutive activity has been demonstrated for other histamine receptor family members (89-92) but in the steady state GTPase assay, the H_4R shows the highest amount of constitutive activity compared with H_1R-H_3R (88).

8.1. Calcium mobilization

Histamine induces calcium release from intracellular calcium stores in both mast cells and eosinophils (65), which was antagonized by thioperamide

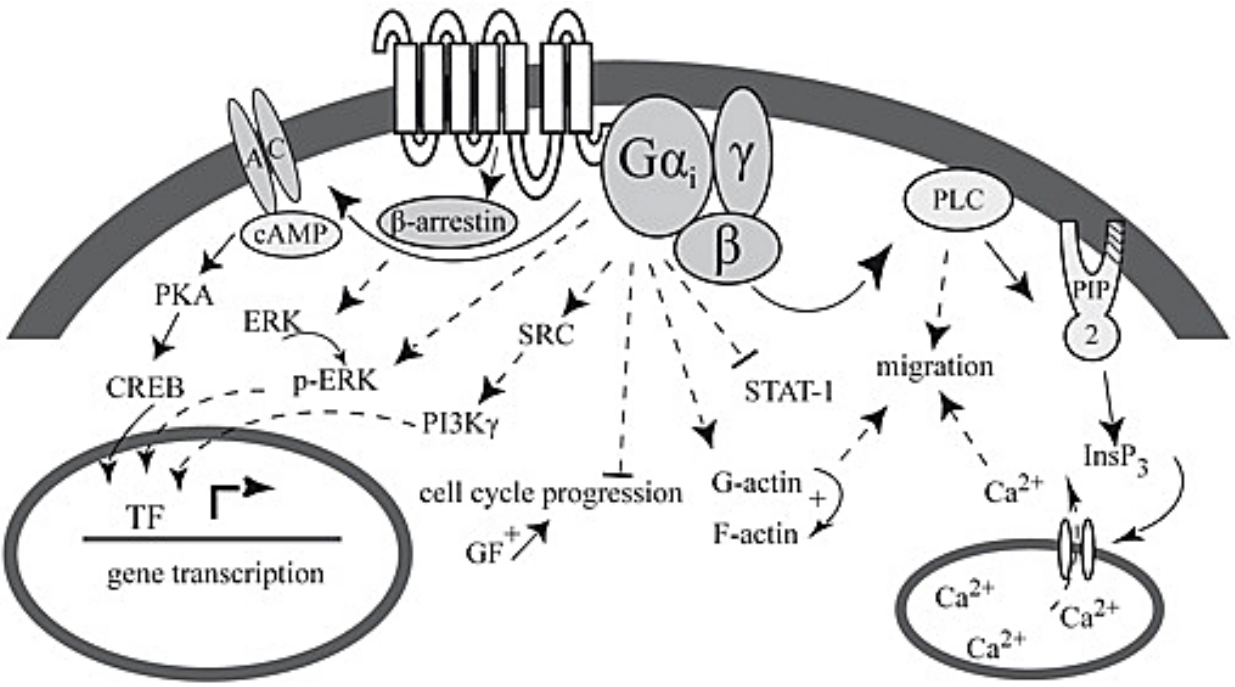

Figure 8. Overview of H_4R signaling pathways in both endogenous and transfected cells. H_4R can signal constitutively, but can also be regulated by agonists, inverse agonists and antagonists. The H_4R activates $Galpha_{i/o}$ and/or beta-arrestin proteins and their downstream signaling pathways.

indicating a role for H_4Rs (64). Direct proof of H_4R-mediated calcium mobilization came from experiments with H_4R knock out mice, as mast cells isolated from these mice failed to mobilize calcium in response to 30 mM histamine (65). Upon histamine binding, H_4R couples to PTX-sensitive $Galpha_{i/o}$ proteins which subsequently can activate phospholipase C (PLC) via their Gbetagamma subunits. PLC hydrolyzes phosphatidylinositol 4,5-biphosphate (PIP2) to diacylglycerol and inositol 1,4,5-triphosphate (InsP3). InsP3 can bind to its receptor on the endoplasmatic reticulum (ER) membrane, thereby stimulating the release of calcium, which can on its turn induce chemotaxis towards histamine (65) (Figure. 8).

8.2. Migration

More than 25 years before the identification of H_4R, Clark and colleagues already observed that low concentrations of histamine induced chemotaxis of eosinophils (93). Soon after the introduction of the newest member of the histamine receptor family a role for H_4R in this eosinophil migration was discovered (94). More recently, the chemotactic properties of isolated human eosinophils were tested in the presence of several H_1R/H_3R agonists, but only histamine could provoke a response. Interestingly, only H_3R/H_4R antagonists were able to block this effect. Since eosinophils do not express H_3R, the H_4R is responsible for the histamine-induced migration of eosinophils (94-96).

The actin cytoskeleton determines both cell morphology and cell movement. The real "moving" is occurring because the G actin monomers are rearranged to form F actin polymers. An imaging approach was used to show that stimulation of H_4R-expressing eosinophils induces maximal actin polymerizaton to F actin already after 5-10 seconds (64, 96). Histamine-induced actin polymerization was inhibited by JNJ 7777120 and H_3R/H_4R inverse agonist thioperamide, confirming an exclusive role for H_4Rs (96). H_4R agonists also induced F-actin polymerization via H_4Rs in MoDC as shown by the observation that clobenpropit-induced polymerization could be blocked by JNJ 7777120 (38).

In addition, H_4R agonists induce a rapid PTX-sensitive shape change in eosinophils, which was blocked by H_4R antagonists, but not by antagonists of other histamine receptors (64). In comparison to the chemokine eotaxin (CCL11)-induced eosinophil migration, histamine was found to be a relative weak chemotactic factor in a whole blood migration assay (95). Of concern is the observation that histamine failed to stimulate guinea-pig eosinophils in a variety of these shape change or chemotaxis assays (64). This again emphasizes the importance of understanding the species differences in H_4R pharmacology.

Histamine stimulation of the H_4R also induces mast cell migration *in vitro*. Investigation of the signaling components proofed that both $Galpha_{i/o}$ and PLC are involved in the downstream signaling events. Histamine inhalation led to an increase in the total number of mast cells and sub-epithelial mast cells in trachea of mice, which could be inhibited by JNJ 7777120 (69), thereby ruling out H_1R and H_2R-mediated effects.

In an *ex vivo* migration assay, skin dendritic cells isolated from mice and guinea pig were able to the enhance chemotaxis upon stimulation with histamine or H_3R/H_4R ligand clobenpropit. These observations were confirmed in *in vitro* migration experiments with bone marrow derived dendritic cells from human and mice (29). This histamine/clobenpropit-induced effect was fully inhibited by JNJ 7777120. However, the histamine-induced chemotaxis could also be fully inhibited by H_1R antagonist diphenhydramine (29), suggesting crosstalk between these two histamine receptor subtypes.

In a human epidermis and murine *in vivo* assay it was shown that Langerhans cell migration from the epidermis was increased upon H_4R activation. In addition, a downregulation of the production of CCL2 was observed (37).

More recently the effect of histamine on migration of human fetal lung fibroblasts to human plasma fibronectin (HFn) *in vitro* was examined (97). Histamine did not induce migration of these cells, but appeared to potentiate the migration to Hfn in a bell shaped relation. Addition of JNJ 7777120 inhibited this effect, as did PTX, indicating an $H_4R/Galpha_{i/o}$-mediated process (Figure. 8) (97).

8.3. Modulation of protein expression and physiological effects regulated by H_4R

Leukocyte chemoattractants affect the function and cell surface expression of adhesion molecules, which play an important role in the interaction of leukocytes with the microvascular endothelium. Histamine is also able to upregulate adhesion molecules (e.g. CD11b/CD18 and CD54) in eosinophils. This upregulation occurs already within 10 min after stimulation (95). Upregulation of CD11b could be inhibited by preincubation with thioperamide (64) indicating a role for the H_4R. In addition, pre-stimulation with histamine increased the amount of eosinophils to migrate towards CCL11, which could also be inhibited by thioperamide (64).

A potentiation is also observed for CXCL12 chemotactic activity on the precursor mast cell population upon stimulation with histamine or supernatants from IgE-activated mast cells. Small interfering RNA (siRNA) was used to specifically block each histamine receptor subtype. This resulted in the identification of the H_4R as the one responsible for the observed synergy. In addition, JNJ 7777120 was able to completely block this potentiation. Interestingly, CXCR4 (receptor for CXCL12) protein levels did not change. An explanation was put forward, in which the authors speculated on a shared signaling pathway between H_4R and CXCR4 downstream of the $Galpha_{i/o}$ protein (98).

Histamine suppresses polyIC-induced IL-12p70 production of MoDC via different pathways activated by H_2R and H_4R. H_4R activates the MAPK pathway, resulting in the activation of AP-1 (Figure. 8). Interestingly, this was independent of ERK1/2 phosphorylation. AP-1 induction by clobenpropit could be blocked by JNJ 7777120, demonstrating that AP-1 is indeed induced by H_4R stimulation. The H_2R-mediated IL-12p70 suppression involves cAMP production (38).

A recent study in peripheral blood mononuclear cell (PBMC) cultures from non-atopic human volunteers showed a possible role for the H_4R in modulating signaling pathways via STAT1. It was already shown that H_4R might play a role in lymphocyte signaling and in Th2 differentiation (69, 99, 100). JNJ 7777120 stimulated the production of STAT1alpha and its downstream phosphorylation in the non-atopic group. JNJ 7777120 could also enhance the binding of STAT1 to DNA. A model was proposed, in which histamine acts through the H_4R on T cells, thereby inhibiting STAT1 activation and thus helps to drive the Th2 polarization by inhibiting IFN-gamma mediated events (101, 102).

Histamine-free histidine decarboxylase deficient mice (HDC-/-) show a functional deficit in invariant natural killer (iNKT) cells. This is clearly demonstrated by decreased IL-4 and IFN-gamma production. Addition of histamine induced a functional recovery that is mediated by the H_4R, since JNJ 7777120 could prevent this. To unambiguously state that the H_4R is involved, iNKT cells of H_4R knock out mice were also tested. These cells generate lower amounts of circulating cytokines than WT mice, clearly showing a role for H_4Rs in this process (103).

H_4R proteins were recently shown to be involved in the production of interleukin 6 (IL-6) from mouse bone marrow-derived mast cells. Both histamine and H_4R agonist JNJ 28610244 can induce the transient production of this cytokine. In turn, H_4R antagonists could inhibit this effect. Additionally, H_4R potentiates the prolonged lipopolysaccharide- (LPS) induced IL-6 production via MAPK (ERK) and Src/PI3Kgamma pathways, suggesting crosstalk between toll-like receptors (TLR) and H_4R signaling pathways (104).

Murine and human progenitor cell populations express functional H_4R. Upon activation of the receptor, the cells show a reduced growth factor-induced cell cycle progression. As a consequence, myeloid, erythroid and lymphoid colony formation is decreased, hence the cells show reduced proliferation. The H_4R thus prevents induction of cell cycle genes, presumably via its cAMP and subsequent protein kinase A (PKA) pathway (Figure. 8). A special role for the H_4R was confirmed with H_4R selective antagonists that restored cell cycle progression. Very interesting is the observation that the arrest of growth factor-induced G1/S phase transition (quiescence) protects the murine and human progenitor cells from the toxicity of the cell cycle dependent anticancer drug Ara-C *in vitro* and reduces aplasia in a murine model of chemotherapy (103). This opens a new possible role for H_4R targeting drugs (105).

The function of H_4R in the stomach was explored in ulcer models in both rat and mice. JNJ 7777120 (10-30 mg/kg sc) reduced the indomethacin-induced gastric mucosal damage by approximately 70% in the conscious

rat. In addition, JNJ 7777120 reduced indomethacin- and bethanechol-induced gastrolesive effects in conscious mice. H_4R agonist VUF 10460 (4- (4-methylpiperazin-1-yl)-6-phenylpyrimidin-2-amine) reduced indomethacin-induced lesions in the rat, but not in mice (106).

8.4. Beta-arrestin recruitment to H_4R / Biased agonism

In addition to G protein coupling, both histamine and 4-methylhistamine induce beta-arrestin recruitment to H_4R as revealed in a protein-fragment complementation-based beta-arrestin recruitment assay (i.e. Tango assay Invitrogen) . Histamine-induced beta-arrestin recruitment to the H_4R was recently confirmed by Rosethorne and Charlton in Pathhunter™ U2OS-H4 / beta-arrestin cells (107). Interestingly, this recruitment was PTX insensitive, indicating a $Galpha_i$-independent mechanism. Surprisingly, the antagonist JNJ 7777120 was shown to behave as a partial agonist by inducing $Galpha_i$-independent beta-arrestin recruitment to the H_4R (71).

Moreover, in the same cell line both histamine and JNJ 7777120 stimulate phosphorylation of ERK in time frames that are characteristic for G protein-mediated (seconds) or beta-arrestin-mediated (~20min) signaling, respectively (Figure. 8) (71).

These recent findings indicate that the H4R ligands may display distinct efficacies towards G protein-dependent and –independent pathways, with the reference antagonist JNJ 7777120 turning out to be a partial agonist with bias towards beta-arrestin driven pathways. Although this ligand-directed signaling is very exciting and opens the door for the development of pathway-selective compounds it also invites to re-assess the efficacies of known H4R ligands towards G-protein-independent pathways (70, 71, 107).

9. FINAL REMARKS

There is substantial evidence that H4R plays a role in inflammatory processes, based on their localization pattern on cells of hematopoietic origin and the medicinal in vivo studies so far. H4R inhibitors can be interesting drugs to counteract allergic reactions, hence the interest of the pharmaceutical industry. However, recently also other expression patterns were identified, such as the brain. In addition, a link between H4R and diseases as rheumatoid arthritis (108), colon (109, 110) and breast cancer (111) was postulated. This opens doors for even more possibilities than the initial anti-inflammatory properties.

New development programs for the search for novel H4R ligands are ongoing. In parallel with the computational design of ligands, and our increased understanding of receptor-ligand binding interactions the ultimate goal is to develop H4R-specific ligands with on forehand known efficacies and binding modes.

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11. REFERENCES

1. W. Vogt. Ber. dtsch. chem. Ges., 40 (1907)

2. G. Barger and H. H. Dale: Chemical structure and sympathomimetic action of amines. *J Physiol*, 41 (1-2), 19-59 (1910)

3. H. H. Dale and P. P. Laidlaw: The physiological action of beta-iminazolylethylamine. *J Physiol*, 41 (5), 318-44 (1910)

4. J. P. Kinet: The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. *Annu Rev Immunol*, 17, 931-72 (1999)

5. A. S. Ash and H. O. Schild: Receptors mediating some actions of histamine. *Br J Pharmacol Chemother*, 27 (2), 427-39 (1966)

6. J. W. Black, W. A. Duncan, C. J. Durant, C. R. Ganellin and E. M. Parsons: Definition and antagonism of histamine H 2 -receptors. *Nature*, 236 (5347), 385-90 (1972)

7. J. M. Arrang, M. Garbarg and J. C. Schwartz: Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. *Nature*, 302 (5911), 832-7 (1983)

8. R. Leurs, R. C. Vollinga and H. Timmerman: The medicinal chemistry and therapeutic potentials of ligands of the histamine H3 receptor. *Prog Drug Res*, 45, 107-65 (1995)

9. T. A. Esbenshade, K. E. Browman, R. S. Bitner, M. Strakhova, M. D. Cowart and J. D. Brioni: The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. *Br J Pharmacol*, 154 (6), 1166-81 (2008)

10. T. W. Lovenberg, B. L. Roland, S. J. Wilson, X. Jiang, J. Pyati, A. Huvar, M. R. Jackson and M. G. Erlander: Cloning and functional expression of the human histamine H3 receptor. *Mol Pharmacol*, 55 (6), 1101-7 (1999)

11. T. Nakamura, H. Itadani, Y. Hidaka, M. Ohta and K. Tanaka: Molecular cloning and characterization of a new human histamine receptor, HH4R. *Biochem Biophys Res Commun*, 279 (2), 615-20 (2000)

12. T. Oda and S. Matsumoto: Identification and characterization of histamine H4 receptor. *Nippon Yakurigaku Zasshi*, 118 (1), 36-42 (2001)

13. T. Nguyen, D. A. Shapiro, S. R. George, V. Setola, D. K. Lee, R. Cheng, L. Rauser, S. P. Lee, K. R. Lynch, B. L. Roth and B. F. O'Dowd: Discovery of a novel member of the histamine receptor family. *Mol Pharmacol*, 59 (3), 427-33 (2001)

14. Y. Zhu, D. Michalovich, H. Wu, K. B. Tan, G. M. Dytko, I. J. Mannan, R. Boyce, J. Alston, L. A. Tierney, X.

Li, N. C. Herrity, L. Vawter, H. M. Sarau, R. S. Ames, C. M. Davenport, J. P. Hieble, S. Wilson, D. J. Bergsma and L. R. Fitzgerald: Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol Pharmacol*, 59 (3), 434-41 (2001)

15. K. L. Morse, J. Behan, T. M. Laz, R. E. West, Jr., S. A. Greenfeder, J. C. Anthes, S. Umland, Y. Wan, R. W. Hipkin, W. Gonsiorek, N. Shin, E. L. Gustafson, X. Qiao, S. Wang, J. A. Hedrick, J. Greene, M. Bayne and F. J. Monsma, Jr.: Cloning and characterization of a novel human histamine receptor. *J Pharmacol Exp Ther*, 296 (3), 1058-66 (2001)

16. C. Liu, X. Ma, X. Jiang, S. J. Wilson, C. L. Hofstra, J. Blevitt, J. Pyati, X. Li, W. Chai, N. Carruthers and T. W. Lovenberg: Cloning and pharmacological characterization of a fourth histamine receptor (H (4)) expressed in bone marrow. *Mol Pharmacol*, 59 (3), 420-6 (2001)

17. F. Coge, S. P. Guenin, H. Rique, J. A. Boutin and J. P. Galizzi: Structure and expression of the human histamine H4-receptor gene. *Biochem Biophys Res Commun*, 284 (2), 301-9 (2001)

18. B. Yu, Y. Shao, P. Li, J. Zhang, Q. Zhong, H. Yang, X. Hu, B. Chen, X. Peng, Q. Wu, Y. Chen, M. Guan, J. Wan and W. Zhang: Copy number variations of the human histamine H4 receptor gene are associated with systemic lupus erythematosus. *Br J Dermatol*, 163 (5), 935-40 (2010)

19. P. Wiedemann, H. Bonisch, F. Oerters and M. Bruss: Structure of the human histamine H3 receptor gene (HRH3) and identification of naturally occurring variations. *J Neural Transm*, 109 (4), 443-53 (2002)

20. M. D. De Backer, I. Loonen, P. Verhasselt, J. M. Neefs and W. H. Luyten: Structure of the human histamine H1 receptor gene. *Biochem J*, 335 (Pt 3), 663-70 (1998)

21. H. Murakami, G. H. Sun-Wada, M. Matsumoto, T. Nishi, Y. Wada and M. Futai: Human histamine H2 receptor gene: multiple transcription initiation and tissue-specific expression. *FEBS Lett*, 451 (3), 327-31 (1999)

22. R. M. van Rijn, A. van Marle, P. L. Chazot, E. Langemeijer, Y. Qin, F. C. Shenton, H. D. Lim, O. P. Zuiderveld, K. Sansuk, M. Dy, M. J. Smit, C. P. Tensen, R. A. Bakker and R. Leurs: Cloning and characterization of dominant negative splice variants of the human histamine H4 receptor. *Biochem J*, 414 (1), 121-31 (2008)

23. B. Yu, Y. Shao, J. Zhang, X. L. Dong, W. L. Liu, H. Yang, L. Liu, M. H. Li, C. F. Yue, Z. Y. Fang, C. Zhang, X. P. Hu, B. C. Chen, Q. Wu, Y. W. Chen, W. Zhang and J. Wan: Polymorphisms in human histamine receptor H4 gene are associated with atopic dermatitis. *Br J Dermatol*, 162 (5), 1038-43 (2010)

24. C. Liu, S. J. Wilson, C. Kuei and T. W. Lovenberg: Comparison of human, mouse, rat, and guinea pig histamine H4 receptors reveals substantial pharmacological species variation. *J Pharmacol Exp Ther*, 299 (1), 121-30 (2001)

25. W. Jiang, H. D. Lim, M. Zhang, P. Desai, H. Dai, P. M. Colling, R. Leurs and R. L. Thurmond: Cloning and pharmacological characterization of the dog histamine H4 receptor. *Eur J Pharmacol*, 592 (1-3), 26-32 (2008)

26. T. Oda, S. Matsumoto, M. Matsumoto, J. Takasaki, M. Kamohara, T. Soga, H. Hiyama, M. Kobori and M. Katoh: Molecular cloning of monkey histamine H4 receptor. *J Pharmacol Sci*, 98 (3), 319-22 (2005)

27. T. Oda, S. Matsumoto, Y. Masuho, J. Takasaki, M. Matsumoto, M. Kamohara, T. Saito, T. Ohishi, T. Soga, H. Hiyama, H. Matsushime and K. Furuichi: cDNA cloning and characterization of porcine histamine H4 receptor. *Biochim Biophys Acta*, 1575 (1-3), 135-8 (2002)

28. H. D. Lim, C. de Graaf, W. Jiang, P. Sadek, P. M. McGovern, E. P. Istyastono, R. A. Bakker, I. J. de Esch, R. L. Thurmond and R. Leurs: Molecular determinants of ligand binding to H4R species variants. *Mol Pharmacol*, 77 (5), 734-43 (2010)

29. W. Baumer, S. Wendorff, R. Gutzmer, T. Werfel, D. Dijkstra, P. Chazot, H. Stark and M. Kietzmann: Histamine H4 receptors modulate dendritic cell migration through skin--immunomodulatory role of histamine. *Allergy*, 63 (10), 1387-94 (2008)

30. D. Dijkstra, R. Leurs, P. Chazot, F. C. Shenton, H. Stark, T. Werfel and R. Gutzmer: Histamine downregulates monocyte CCL2 production through the histamine H4 receptor. *J Allergy Clin Immunol*, 120 (2), 300-7 (2007)

31. D. Dijkstra, H. Stark, P. L. Chazot, F. C. Shenton, R. Leurs, T. Werfel and R. Gutzmer: Human inflammatory dendritic epidermal cells express a functional histamine H4 receptor. *J Invest Dermatol*, 128 (7), 1696-703 (2008)

32. R. M. van Rijn, P. L. Chazot, F. C. Shenton, K. Sansuk, R. A. Bakker and R. Leurs: Oligomerization of recombinant and endogenously expressed human histamine H (4) receptors. *Mol Pharmacol*, 70 (2), 604-15 (2006)

33. G. Morini, G. Becchi, F. C. Shenton, P. L. Chazot and D. Grandi: Histamine H3 and H4 receptors are expressed on distinct endocrine cell types in the rat fundic mucosa. *Inflamm Res, 57* Suppl 1, S57-8 (2008)

34. L. E. Sander, A. Lorentz, G. Sellge, M. Coeffier, M. Neipp, T. Veres, T. Frieling, P. N. Meier, M. P. Manns and S. C. Bischoff: Selective expression of histamine receptors H1R, H2R, and H4R, but not H3R, in the human intestinal tract. *Gut,* 55 (4), 498-504 (2006)

35. W. M. Connelly, F. C. Shenton, N. Lethbridge, R. Leurs, H. J. Waldvogel, R. L. Faull, G. Lees and P. L. Chazot: The histamine H4 receptor is functionally

expressed on neurons in the mammalian CNS. *Br J Pharmacol*, 157 (1), 55-63 (2009)

36. T. Oda, N. Morikawa, Y. Saito, Y. Masuho and S. Matsumoto: Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J Biol Chem*, 275 (47), 36781-6 (2000)

37. M. Gschwandtner, K. Rossbach, D. Dijkstra, W. Baumer, M. Kietzmann, H. Stark, T. Werfel and R. Gutzmer: Murine and human Langerhans cells express a functional histamine H4 receptor: modulation of cell migration and function. Allergy, 65 (7), 840-9

38. R. Gutzmer, C. Diestel, S. Mommert, B. Kother, H. Stark, M. Wittmann and T. Werfel: Histamine H4 receptor stimulation suppresses IL-12p70 production and mediates chemotaxis in human monocyte-derived dendritic cells. *J Immunol*, 174 (9), 5224-32 (2005)

39. N. L. Lethbridge and P. L. Chazot: Immunological identification of the mouse H4 histamine receptor on spinal cord motor neurons using a novel anti-mouse H4R antibody. *Inflamm Res*, 59 Suppl 2, S197-8

40. M. Nakaya, N. Takeuchi and K. Kondo: Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. *Ann Otol Rhinol Laryngol*, 113 (7), 552-7 (2004)

41. K. Boer, E. Helinger, A. Helinger, P. Pocza, Z. Pos, P. Demeter, Z. Baranyai, K. Dede, Z. Darvas and A. Falus: Decreased expression of histamine H1 and H4 receptors suggests disturbance of local regulation in human colorectal tumours by histamine. *Eur J Cell Biol*, 87 (4), 227-36 (2008)

42. C. de Graaf, N. Foata, O. Engkvist and D. Rognan: Molecular modeling of the second extracellular loop of G-protein coupled receptors and its implication on structure-based virtual screening. *Proteins-Structure Function and Bioinformatics*, 71 (2), 599-620 (2008)

43. Ballesteros and Weinstein. *Methods Neurosci*, 25, 366-428 (1995)

44. K. Kaupmann, B. Malitschek, V. Schuler, J. Heid, W. Froestl, P. Beck, J. Mosbacher, S. Bischoff, A. Kulik, R. Shigemoto, A. Karschin and B. Bettler: GABA (B)-receptor subtypes assemble into functional heteromeric complexes. *Nature*, 396 (6712), 683-7 (1998)

45. J. H. White, A. Wise, M. J. Main, A. Green, N. J. Fraser, G. H. Disney, A. A. Barnes, P. Emson, S. M. Foord and F. H. Marshall: Heterodimerization is required for the formation of a functional GABA (B) receptor. *Nature*, 396 (6712), 679-82 (1998)

46. K. A. Jones, B. Borowsky, J. A. Tamm, D. A. Craig, M. M. Durkin, M. Dai, W. J. Yao, M. Johnson, C. Gunwaldsen, L. Y. Huang, C. Tang, Q. Shen, J. A. Salon, K. Morse, T. Laz, K. E. Smith, D. Nagarathnam, S. A. Noble, T. A. Branchek and C. Gerald: GABA (B) receptors function as a heteromeric assembly of the subunits GABA (B)R1 and GABA (B)R2. *Nature*, 396 (6712), 674-9 (1998)

47. J. P. Pin, J. Kniazeff, V. Binet, J. Liu, D. Maurel, T. Galvez, B. Duthey, M. Havlickova, J. Blahos, L. Prezeau and P. Rondard: Activation mechanism of the heterodimeric GABA (B) receptor. Biochem Pharmacol, 68 (8), 1565-72 (2004)

48. H. F. Vischer, A. O. Watts, S. Nijmeijer and R. Leurs: G protein-coupled receptors: walking hand-in-hand, talking hand-in-hand? Br J Pharmacol, 163 (2), 246-60 (2011)

49. J. J. Carrillo, J. Pediani and G. Milligan: Dimers of class A G protein-coupled receptors function via agonist-mediated trans-activation of associated G proteins. *J Biol Chem*, 278 (43), 42578-87 (2003)

50. R. A. Bakker, G. Dees, J. J. Carrillo, R. G. Booth, J. F. Lopez-Gimenez, G. Milligan, P. G. Strange and R. Leurs: Domain swapping in the human histamine H1 receptor. *J Pharmacol Exp Ther*, 311 (1), 131-8 (2004)

51. Y. Fukushima, T. Asano, T. Saitoh, M. Anai, M. Funaki, T. Ogihara, H. Katagiri, N. Matsuhashi, Y. Yazaki and K. Sugano: Oligomer formation of histamine H2 receptors expressed in Sf9 and COS7 cells. *FEBS Lett*, 409 (2), 283-6 (1997)

52. F. C. Shenton, V. Hann and P. L. Chazot: Evidence for native and cloned H3 histamine receptor higher oligomers. *Inflamm Res*, 54 Suppl 1, S48-9 (2005)

53. S. Nijmeijer, R. Leurs, M. J. Smit and H. F. Vischer: The Epstein-Barr virus-encoded G protein-coupled receptor BILF1 hetero-oligomerizes with human CXCR4, scavenges Galphai proteins, and constitutively impairs CXCR4 functioning. *J Biol Chem*, 285 (38), 29632-41 (2010)

54. H. D. Lim, R. M. van Rijn, P. Ling, R. A. Bakker, R. L. Thurmond and R. Leurs: Evaluation of histamine H1-, H2-, and H3-receptor ligands at the human histamine H4 receptor: identification of 4-methylhistamine as the first potent and selective H4 receptor agonist. *J Pharmacol Exp Ther*, 314 (3), 1310-21 (2005)

55. I. J. de Esch, R. L. Thurmond, A. Jongejan and R. Leurs: The histamine H4 receptor as a new therapeutic target for inflammation. *Trends Pharmacol Sci*, 26 (9), 462-9 (2005)

56. R. A. Smits, I. J. de Esch, O. P. Zuiderveld, J. Broeker, K. Sansuk, E. Guaita, G. Coruzzi, M. Adami, E. Haaksma and R. Leurs: Discovery of quinazolines as histamine H4 receptor inverse agonists using a scaffold hopping approach. *J Med Chem*, 51 (24), 7855-65 (2008)

57. R. A. Smits, H. D. Lim, B. Stegink, R. A. Bakker, I. J. de Esch and R. Leurs: Characterization of the histamine H4 receptor binding site. Part 1. Synthesis and

pharmacological evaluation of dibenzodiazepine derivatives. *J Med Chem*, 49 (15), 4512-6 (2006)

58. H. D. Lim, R. A. Smits, R. A. Bakker, C. M. van Dam, I. J. de Esch and R. Leurs: Discovery of S- (2-guanidylethyl)-isothiourea (VUF 8430) as a potent nonimidazole histamine H4 receptor agonist. *J Med Chem*, 49 (23), 6650-1 (2006)

59. T. Hashimoto, S. Harusawa, L. Araki, O. P. Zuiderveld, M. J. Smit, T. Imazu, S. Takashima, Y. Yamamoto, Y. Sakamoto, T. Kurihara, R. Leurs, R. A. Bakker and A. Yamatodani: A selective human H (4)-receptor agonist: (-)-2-cyano-1-methyl-3- ((2R,5R)-5- (1H-imidazol-4 (5)-yl)tetrahydrofuran-2-y) methylguanidine. *J Med Chem*, 46 (14), 3162-5 (2003)

60. P. Igel, R. Geyer, A. Strasser, S. Dove, R. Seifert and A. Buschauer: Synthesis and structure-activity relationships of cyanoguanidine-type and structurally related histamine H4 receptor agonists. *J Med Chem*, 52 (20), 6297-313 (2009)

61. P. Igel, S. Dove and A. Buschauer: Histamine H4 receptor agonists. *Bioorg Med Chem Lett*, 20 (24), 7191-9 (2010)

62. F. W. Yu, R L; Wei, J; Desai, P J; McGovern, P M; Dunford, P J; Karlsson, L; Thurmond, R L: Pharmacological characterization of oxime agonists of the histamine H4 receptor. *Journal of Receptor, Ligand and Channel Research*, 3, 37-49 (2010)

63. B. M. Savall, J. P. Edwards, J. D. Venable, D. J. Buzard, R. Thurmond, M. Hack and P. McGovern: Agonist/antagonist modulation in a series of 2-aryl benzimidazole H4 receptor ligands. *Bioorg Med Chem Lett*, 20 (11), 3367-71 (2010)

64. K. F. Buckland, T. J. Williams and D. M. Conroy: Histamine induces cytoskeletal changes in human eosinophils via the H (4) receptor. *Br J Pharmacol*, 140 (6), 1117-27 (2003)

65. C. L. Hofstra, P. J. Desai, R. L. Thurmond and W. P. Fung-Leung: Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. *J Pharmacol Exp Ther*, 305 (3), 1212-21 (2003)

66. K. Takeshita, K. B. Bacon and F. Gantner: Critical role of L-selectin and histamine H4 receptor in zymosan-induced neutrophil recruitment from the bone marrow: comparison with carrageenan. *J Pharmacol Exp Ther*, 310 (1), 272-80 (2004)

67. B. B. Damaj, C. B. Becerra, H. J. Esber, Y. Wen and A. A. Maghazachi: Functional expression of H4 histamine receptor in human natural killer cells, monocytes, and dendritic cells. *J Immunol*, 179 (11), 7907-15 (2007)

68. J. A. Jablonowski, C. A. Grice, W. Chai, C. A. Dvorak, J. D. Venable, A. K. Kwok, K. S. Ly, J. Wei, S. M. Baker, P. J. Desai, W. Jiang, S. J. Wilson, R. L. Thurmond, L. Karlsson, J. P. Edwards, T. W. Lovenberg and N. I. Carruthers: The first potent and selective non-imidazole human histamine H4 receptor antagonists. *J Med Chem*, 46 (19), 3957-60 (2003)

69. R. L. Thurmond, P. J. Desai, P. J. Dunford, W. P. Fung-Leung, C. L. Hofstra, W. Jiang, S. Nguyen, J. P. Riley, S. Sun, K. N. Williams, J. P. Edwards and L. Karlsson: A potent and selective histamine H4 receptor antagonist with anti-inflammatory properties. *J Pharmacol Exp Ther*, 309 (1), 404-13 (2004)

70. R. Seifert, E. H. Schneider, S. Dove, I. Brunskole, D. Neumann, A. Strasser and A. Buschauer: Paradoxical stimulatory effects of the "standard" histamine H4-receptor antagonist JNJ7777120: The H4-receptor joins the club of 7TM receptors exhibiting functional selectivity. *Mol Pharmacol* (2011)

71. E. M. Rosethorne and S. J. Charlton: Agonist-biased signaling at the histamine H4 receptor: JNJ7777120 recruits beta-arrestin without activating G proteins. *Mol Pharmacol*, 79 (4), 749-57 (2011)

72. N. Terzioglu, R. M. van Rijn, R. A. Bakker, I. J. De Esch and R. Leurs: Synthesis and structure-activity relationships of indole and benzimidazole piperazines as histamine H (4) receptor antagonists. *Bioorg Med Chem Lett*, 14 (21), 5251-6 (2004)

73. J. D. Venable, H. Cai, W. Chai, C. A. Dvorak, C. A. Grice, J. A. Jablonowski, C. R. Shah, A. K. Kwok, K. S. Ly, B. Pio, J. Wei, P. J. Desai, W. Jiang, S. Nguyen, P. Ling, S. J. Wilson, P. J. Dunford, R. L. Thurmond, T. W. Lovenberg, L. Karlsson, N. I. Carruthers and J. P. Edwards: Preparation and biological evaluation of indole, benzimidazole, and thienopyrrole piperazine carboxamides: potent human histamine h (4) antagonists. *J Med Chem*, 48 (26), 8289-98 (2005)

74. H. Liu, R. J. Altenbach, T. L. Carr, P. Chandran, G. C. Hsieh, L. G. Lewis, A. M. Manelli, I. Milicic, K. C. Marsh, T. R. Miller, M. I. Strakhova, T. A. Vortherms, B. D. Wakefield, J. M. Wetter, D. G. Witte, P. Honore, T. A. Esbenshade, J. D. Brioni and M. D. Cowart: cis-4-(Piperazin-1-yl)-5,6,7a,8,9,10,11,11a-octahydrobenzofuro (2,3-h)quina zolin-2-amine (A-987306), a new histamine H4R antagonist that blocks pain responses against carrageenan-induced hyperalgesia. *J Med Chem*, 51 (22), 7094-8 (2008)

75. W. C. Lumma, Jr., R. D. Hartman, W. S. Saari, E. L. Engelhardt, V. J. Lotti and C. A. Stone: Piperazinylquinoxalines with central serotoninmimetic activity. *J Med Chem*, 24 (1), 93-101 (1981)

76. R. A. Smits, H. D. Lim, A. Hanzer, O. P. Zuiderveld, E. Guaita, M. Adami, G. Coruzzi, R. Leurs and I. J. de Esch: Fragment based design of new H4 receptor-ligands with anti-inflammatory properties *in vivo*. *J Med Chem*, 51 (8), 2457-67 (2008)

77. R. A. Smits, M. Adami, E. P. Istyastono, O. P. Zuiderveld, C. M. van Dam, F. J. de Kanter, A. Jongejan, G. Coruzzi, R. Leurs and I. J. de Esch: Synthesis and QSAR of quinazoline sulfonamides as highly potent human histamine H4 receptor inverse agonists. *J Med Chem*, 53 (6), 2390-400 (2010)

78. R. A. Smits, R. Leurs and I. J. de Esch: Major advances in the development of histamine H4 receptor ligands. *Drug Discov Today*, 14 (15-16), 745-53 (2009)

79. H. Engelhardt, R. A. Smits, R. Leurs, E. Haaksma and I. J. de Esch: A new generation of anti-histamines: Histamine H4 receptor antagonists on their way to the clinic. *Curr Opin Drug Discov Devel*, 12 (5), 628-43 (2009)

80. H. D. Lim, A. Jongejan, R. A. Bakker, E. Haaksma, I. J. de Esch and R. Leurs: Phenylalanine 169 in the second extracellular loop of the human histamine H4 receptor is responsible for the difference in agonist binding between human and mouse H4 receptors. *J Pharmacol Exp Ther*, 327 (1), 88-96 (2008)

81. J. S. Surgand, J. Rodrigo, E. Kellenberger and D. Rognan: A chemogenomic analysis of the transmembrane binding cavity of human G-protein-coupled receptors. *Proteins-Structure Function and Bioinformatics*, 62 (2), 509-538 (2006)

82. E. P. Istyastono, C. de Graaf, I. J. de Esch and R. Leurs: Molecular Determinants of Selective Agonist and Antagonist Binding to the Histamine H (4) Receptor. *Curr Top Med Chem* (2011)

83. A. Jongejan, H. D. Lim, R. A. Smits, I. J. de Esch, E. Haaksma and R. Leurs: Delineation of agonist binding to the human histamine H4 receptor using mutational analysis, homology modeling, and ab initio calculations. *J Chem Inf Model*, 48 (7), 1455-63 (2008)

84. N. Shin, E. Coates, N. J. Murgolo, K. L. Morse, M. Bayne, C. D. Strader and F. J. Monsma, Jr.: Molecular modeling and site-specific mutagenesis of the histamine-binding site of the histamine H4 receptor. *Mol Pharmacol*, 62 (1), 38-47 (2002)

85. L. Shi and J. A. Javitch: The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop. *Annu Rev Pharmacol Toxicol*, 42, 437-67 (2002)

86. E. P. Istyastono, C. de Graaf, I. J. de Esch and R. Leurs: Molecular determinants of selective agonist and antagonist binding to the histamine H receptor. *Curr Top Med Chem*, 11 (6), 661-79 (2011)

87. T. Werner, K. Sander, Y. Tanrikulu, T. Kottke, E. Proschak, H. Stark and G. Schneider: In silico characterization of ligand binding modes in the human histamine H4 receptor and their impact on receptor activation. *Chembiochem*, 11 (13), 1850-5 (2010)

88. E. H. Schneider and R. Seifert: Histamine H (4) receptor-RGS fusion proteins expressed in Sf9 insect cells: a sensitive and reliable approach for the functional characterization of histamine H (4) receptor ligands. *Biochem Pharmacol*, 78 (6), 607-16 (2009)

89. R. A. Bakker, K. Wieland, H. Timmerman and R. Leurs: Constitutive activity of the histamine H (1) receptor reveals inverse agonism of histamine H (1) receptor antagonists. *Eur J Pharmacol*, 387 (1), R5-7 (2000)

90. M. J. Smit, R. Leurs, A. E. Alewijnse, J. Blauw, G. P. Van Nieuw Amerongen, Y. Van De Vrede, E. Roovers and H. Timmerman: Inverse agonism of histamine H2 antagonist accounts for upregulation of spontaneously active histamine H2 receptors. *Proc Natl Acad Sci U S A*, 93 (13), 6802-7 (1996)

91. K. Wieland, G. Bongers, Y. Yamamoto, T. Hashimoto, A. Yamatodani, W. M. Menge, H. Timmerman, T. W. Lovenberg and R. Leurs: Constitutive activity of histamine h (3) receptors stably expressed in SK-N-MC cells: display of agonism and inverse agonism by H (3) antagonists. *J Pharmacol Exp Ther*, 299 (3), 908-14 (2001)

92. S. Morisset, A. Rouleau, X. Ligneau, F. Gbahou, J. Tardivel-Lacombe, H. Stark, W. Schunack, C. R. Ganellin, J. C. Schwartz and J. M. Arrang: High constitutive activity of native H3 receptors regulates histamine neurons in brain. *Nature*, 408 (6814), 860-4 (2000)

93. R. A. Clark, J. I. Gallin and A. P. Kaplan: The selective eosinophil chemotactic activity of histamine. *J Exp Med*, 142 (6), 1462-76 (1975)

94. M. O'Reilly, R. Alpert, S. Jenkinson, R. P. Gladue, S. Foo, S. Trim, B. Peter, M. Trevethick and M. Fidock: Identification of a histamine H4 receptor on human eosinophils--role in eosinophil chemotaxis. *J Recept Signal Transduct Res*, 22 (1-4), 431-48 (2002)

95. P. Ling, K. Ngo, S. Nguyen, R. L. Thurmond, J. P. Edwards, L. Karlsson and W. P. Fung-Leung: Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br J Pharmacol*, 142 (1), 161-71 (2004)

96. R. Barnard, A. Barnard, G. Salmon, W. Liu and S. Sreckovic: Histamine-induced actin polymerization in human eosinophils: an imaging approach for histamine H4 receptor. *Cytometry* A, 73 (4), 299-304 (2008)

97. T. Kohyama, Y. Yamauchi, H. Takizawa, S. Kamitani, S. Kawasaki and T. Nagase: Histamine stimulates human lung fibroblast migration. *Mol Cell Biochem*, 337 (1-2), 77-81

98. V. Godot, M. Arock, G. Garcia, F. Capel, C. Flys, M. Dy, D. Emilie and M. Humbert: H4 histamine receptor mediates optimal migration of mast cell precursors to CXCL12. *J Allergy Clin Immunol*, 120 (4), 827-34 (2007)

99. M. Dy and E. Schneider: Histamine-cytokine connection in immunity and hematopoiesis. *Cytokine Growth Factor Rev*, 15 (5), 393-410 (2004)

100. E. Schneider, M. Rolli-Derkinderen, M. Arock and M. Dy: Trends in histamine research: new functions during immune responses and hematopoiesis. *Trends Immunol*, 23 (5), 255-63 (2002)

101. M. Jutel, T. Watanabe, S. Klunker, M. Akdis, O. A. Thomet, J. Malolepszy, T. Zak-Nejmark, R. Koga, T. Kobayashi, K. Blaser and C. A. Akdis: Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature*, 413 (6854), 420-5 (2001)

102. B. Horr, H. Borck, R. Thurmond, S. Grosch and F. Diel: STAT1 phosphorylation and cleavage is regulated by the histamine (H4) receptor in human atopic and non-atopic lymphocytes. *Int Immunopharmacol*, 6 (10), 1577-85 (2006)

103. M. C. Leite-de-Moraes, S. Diem, M. L. Michel, H. Ohtsu, R. L. Thurmond, E. Schneider and M. Dy: Cutting edge: histamine receptor H4 activation positively regulates *in vivo* IL-4 and IFN-gamma production by invariant NKT cells. *J Immunol*, 182 (3), 1233-6 (2009)

104. P. Desai and R. L. Thurmond: Histamine H (4) receptor activation enhances LPS-induced IL-6 production in mast cells via ERK and PI3K activation. *Eur J Immunol*, 41 (6), 1764-73 (2011)

105. A. F. Petit-Bertron, F. Machavoine, M. P. Defresne, M. Gillard, P. Chatelain, P. Mistry, E. Schneider and M. Dy: H4 histamine receptors mediate cell cycle arrest in growth factor-induced murine and human hematopoietic progenitor cells. *PLoS One*, 4 (8), e6504 (2009)

106. G. A. Coruzzi, M.' Pozzoli, C.; Smits, R.; De Esch, I.; Leurs, R.: Gastroprotective effects of histamine H4 receptor ligands in rodent ulcer models. *Proceedings of the Britisch Pharmacological Society*, 7 (4) (2010)

107. E. M. Rosethorne and S. J. Charlton: Agonist-biased signalling at the histamine H4 receptor: JNJ7777120 recruits beta-arrestin without activating G proteins. *Mol Pharmacol* (2010)

108. Y. Ikawa, M. Suzuki, S. Shiono, E. Ohki, H. Moriya, E. Negishi and K. Ueno: Histamine H4 receptor expression in human synovial cells obtained from patients suffering from rheumatoid arthritis. *Biol Pharm Bull*, 28 (10), 2016-8 (2005)

109. F. Cianchi, C. Cortesini, N. Schiavone, F. Perna, L. Magnelli, E. Fanti, D. Bani, L. Messerini, V. Fabbroni, G. Perigli, S. Capaccioli and E. Masini: The role of cyclooxygenase-2 in mediating the effects of histamine on cell proliferation and vascular endothelial growth factor production in colorectal cancer. *Clin Cancer Res*, 11 (19 Pt 1), 6807-15 (2005)

110. C. Varga, K. Horvath, A. Berko, R. L. Thurmond, P. J. Dunford and B. J. Whittle: Inhibitory effects of histamine H4 receptor antagonists on experimental colitis in the rat. *Eur J Pharmacol*, 522 (1-3), 130-8 (2005)

111. D. Maslinska, M. Laure-Kamionowska, K. T. Maslinski, K. Deregowski, G. Szewczyk and S. Maslinski: Histamine H (4) receptors on mammary epithelial cells of the human breast with different types of carcinoma. *Inflamm Res*, 55 Suppl 1, S77-8 (2006)

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