Modelling and mutation studies on the histamine H₁-receptor agonist binding site reveal different binding modes for H₁-agonists: Asp¹¹⁶ (TM3) has a constitutive role in receptor stimulation

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Summary
A modelling study has been carried out, investigating the binding of histamine (Hist), 2-methylhistamine (2-MeHist) and 2-phenylhistamine (2-PhHist) at two postulated agonistic binding sites on transmembrane domain 5 (TM5) of the histamine H₁-receptor. For this purpose a conformational analysis study was performed on three particular residues of TM5, i.e., Lys²⁰⁰, Thr²⁰³ and Asn²⁰⁷, for which a functional role in binding has been proposed. The most favourable results were obtained for the interaction between Hist and the Lys²⁰⁰/Asn²⁰⁷ pair. Therefore, Lys²⁰⁰ was subsequently mutated and converted to an alanine, resulting in a 50-fold decrease of H₁-receptor stimulation by histamine. Altogether, the data suggest that the Lys²⁰⁰/Asn²⁰⁷ pair is important for activation of the H₁-receptor by histamine. In contrast, analogues of 2-PhHist seem to belong to a distinct subclass of histamine agonists and an alternative mode of binding is proposed in which the 2-phenyl ring binds to the same receptor location as one of the aromatic rings of classical histamine H₁-agonists. Subsequently, the binding modes of the agonists Hist, 2-MeHist and 2-PhHist and the H₁-antagonist cyproheptadine were evaluated in three different seven-α-helical models of the H₁-receptor built in homology with bacteriorhodopsin, but using three different alignments. Our findings suggest that the position of the carboxylate group of Asp¹¹⁶ (TM3) within the receptor pocket depends on whether an agonist or an antagonist binds to the protein; a conformational change of this aspartate residue upon agonist binding is expected to play an essential role in receptor stimulation.

Introduction
Of the many histamine analogues that have been tested on H₁-receptors, histamine (1, Fig. 1) itself appears to be among the most potent and effective H₁-agonists [1]. Potent and highly selective histamine H₁-agonists belong to the class of 2-phenylhistamine analogues [2,3]: compounds 11b and 11c (Fig. 1) have H₁-agonistic activities of 129% and 112% relative to histamine, respectively [4].

In general, several five-membered rings (i.e., 2, 3, 8-10, Fig. 1), and even six-membered rings such as in 2-pyridylethylamine (2-PEA, 4) can replace the imidazole ring of histamine, while retaining H₁-agonist activity [1]. A proximal basic nitrogen (N⁺) appears to be crucial for agonistic activity; it is found in all H₁-agonists with an activity >0.1% of that of histamine (compare 4 with 5 and 6, Fig. 1). Moreover, this proximal nitrogen cannot be methylated without total loss of activity (compare 1 with 7, Fig. 1). Therefore, the bioactive form of histamine at H₁-receptors is most likely the N⁺-H tautomer in which the N⁺ is freely accessible.

Substitution in the imidazole ring at the 2-position with alkyl groups higher than methyl (8) is highly unfavourable for H₁-receptor activity, probably due to steric
Fig. 1. A selection of compounds, illustrating some aspects of the structural properties necessary for H₁-receptor activation (see text). Agonist activities measured on the guinea pig ileum of compounds 2–10 relative to histamine (= 100%) were taken from Cooper et al. [1]. Relative activities of 11a–c were taken from a study by Zingel and Schunack [4]. Compounds 1, 3, 4, 8 and 11c have recently been tested on two mutant H₁-receptors (Thr²³ → Ala²³ and Asn²⁷ → Ala²⁷ [5]). Histamine (1), 2-methylhistamine (8) and 2-phenylhistamine (11a) are investigated in the present theoretical study.

effects [5]. Therefore, at first sight, it is surprising that aromatic groups are tolerated at this position (i.e., 11a–c). However, recent site-directed mutagenesis studies by our group have revealed that 2-phenylhistamine analogues form a distinct subclass of histamine agonists, since they appear to interact differently with Asn²⁷ in TM5 [5].

The aspartic acid in transmembrane domain 3 (TM3), which is conserved in all aminergic receptors (Asp¹¹⁶ in the guinea pig H₁-receptor (gpH₁R) [6]), has been shown to be essential for agonist and antagonist binding at histamine H₁-receptors [7]. For G-protein coupled receptors (GPCRs) that bind aminergic ligands, it is generally accepted that this aspartate interacts with the positively charged basic group of the ligand. In contrast, receptor specificity appears to be defined by residues in other TM domains, especially those in TM5, as has been suggested by mutagenesis studies on the hamster β₁-adrenergic receptor (Ser²⁴ and Ser²⁷ [8]) and the canine H₂-histaminergic receptor (Asp¹⁸⁶ and Thr¹⁹² [9]). Therefore we decided to identify the corresponding functional residues in TM5 of the H₁-receptor that are responsible for binding of the imidazole ring of histamine in its N²-H tautomeric form. Interaction between the imidazole ring and the H₁-receptor is likely to be optimal when both the N²-H and the N⁴ functional groups can form hydrogen bonds with protein residues. Possible hydrogen-bond donor and acceptor functionalities in TM5 are Asn²⁷, as an H-bond accepting moiety for the N²-proton, and either Thr²⁰³ or Lys²⁰⁰ as an H-bond donor for N⁴. The presence of Lys²⁰⁰ in this region of TM5 is remarkable, since in all other aminergic GPCRs no charged residues are observed in the corresponding TM domain (Fig. 2).

In the primary amino acid sequence of the histamine H₁-receptor, a third hydrogen-bond donor residue (Thr²¹²) is observed in the proximity of Asn²⁰⁷ (Fig. 2). However, with respect to the above-mentioned residues Lys²⁰⁰, Thr²¹² and Asn²⁰⁷, residue Thr²¹² is found at the opposite site of the TM5 α-helix (i.e., C⁸(Thr²¹²)-C⁹(Thr²¹²)-C⁰(Thr²¹²)-C⁹(Thr²¹²) = 151°). Since, based on sequence homology with the β₁-adrenergic receptor and the histamine H₂-receptor, Thr²¹² can be expected to be absent from the receptor binding pocket and to point towards the membrane environment, we did not further consider Thr²¹² in our modelling study.

A severe disadvantage of GPCR modelling studies using the cryomicroscopy structure of bacteriorhodopsin [10] as a template is the known low homology between the TM domains of GPCRs and bacteriorhodopsin (6–11% identity). This low homology has resulted in largely different alignments used by various research groups [11–18]. It is therefore evident that the choice of an alignment for GPCR homology building remains fairly arbitrary. As yet, there is no consensus on the relative orientation of the GPCR α-helices. Therefore, docking studies must be interpreted with caution, especially when the interaction between (usually small) agonists and residues in different TM domains is investigated.

In view of these drawbacks, we have chosen a simplified strategy and focussed in detail on the possible interactions of histaminergic ligands with only one TM domain (TM5). The influence of other domains than TM5 on agonistic action was temporarily neglected and TM5 was treated as a separate entity (an oligopeptide containing 10 residues). An extensive conformational analysis of the three candidate residues Lys²⁰⁰, Thr²¹² and Asn²⁰⁷ was performed and their interaction with the imidazole ring of Hist (1), 2-MeHist (8) and 2-PhHist (11a) was studied.