Summary

Histamine is a biogenic amine well-known for its role in allergic reactions. When histamine is released in the body, for example, after specific antigens, such as pollen or dust, come in contact with mast cells, it exerts its effect by binding to histamine receptors. Histamine receptors are G protein-coupled receptors (GPCRs) or seven-transmembrane domain (7TM) receptors, which represent the largest class of membrane-bound proteins in the human genome. Some of the best-selling drugs on the market today such as Allegra® and Claritin® target histamine receptors. So far four subtypes of histamine receptors have been discovered and cloned, and the research described in this thesis is mainly focused on the histamine H₁ receptor (H₁R). Even before the cloning of the human H₁R, scientists have been able to develop antagonists that potently block histamine from binding to the H₁R. Through the years a steady increase in the structural knowledge of the receptor, has led to increases in selectivity and strength of these antagonists, while at the same time led to a reduction in side effects such as sedation. Pharmaceutical companies rely heavily on structure-based design for lead optimization in drug discovery, especially if X-ray crystal structures of a target protein are available. It is widely used in drug discovery for soluble proteins, because they are more straightforward to isolate, purify and crystallize in comparisons to membrane proteins. Despite the importance of membrane proteins such as GPCRs as drug targets, their structure determination has been very limited compared to soluble proteins. The low success rate is due to the difficulties in obtaining large amounts of high quality proteins. The research described in this thesis was aimed to provide a better understanding of GPCR structure in general and of the human H₁R in specific, through large scale production of the H₁R and subsequent purification and structural analysis by mass spectrometry. Chapter 1 gives a brief overview of the current knowledge of GPCRs and H₁R in particular. Chapter 2 provides a systematic and thorough review of the different options available to express and purify GPCRs, including the H₁R protein. In Chapter 3, we described how we have made use of a well established baculovirus/insect cell expression system, and an in vitro cell free expression system to produce large amounts of the human H₁R and H₄R protein. Moreover, this chapter also shows details in the use of immobilized metal affinity chromatography (IMAC) to purify the receptors after production, as well as our endeavors to obtain the highest possible sequence coverage of the two receptors by MALDI-ToF and LC-MS/MS. Having established a successful and reproducible method to obtain large and pure enough quantities of the human H₁R protein allowed us to continue our goal of improving the structural knowledge and binding pocket of the H₁R. In chapter 4, we describe how we employed MS analysis to discover that an asparagine residue in the N-terminus of the H₁R (Asn5) is glycosylated in insect cells, which had
been used in conjunction with the baculovirus/insect cell expression system. Moreover we were able to locate the residue to which phenoxybenzamine, an irreversible H1R antagonist, covalently binds to the H1R by using MS analysis, thereby showing the great potential of our methods in the production, purification and MS analysis of the H1R protein could have on structural analysis of GPCRs. It is known that GPCRs can become activated in the absence of ligands. The degree of this constitutive activity can also be modulated by single point mutations of the receptor. Constitutive activity of receptors can be used to investigate which residues in a GPCR contributes to receptor activation and how. In chapter 5 we used site directed mutagensis of the H1R to identify residues that are involved in activation of the receptor. Our results suggest that T3.37 interacts with TM5 in the inactive state of the receptor, while S3.39 changes conformation upon receptor activation to achieve, together with S3.36, new water-mediated inter-helical interactions with TM2 and TM7 that stabilize the active state. In addition, we provide the first evidence that the hydrophobic side chain at position 3.40, highly conserved in the rhodopsin family of GPCRs, plays a key role in facilitating the agonist-induced relocation of TM5. Mutation of I3.40 to either alanine or glycine, i.e. removing the bulky side chain at this position, abolishes the constitutive activity of the H1R, the effect of constitutive-activity increasing mutations, as well as the histamine-induced receptor activation. In chapter 6 we describe our detailed investigation of a point mutated version of the H1R. Previous research had identified an isoleucine residue at position 6.45 of the H1R to be prone to yield high constitutively active mutants. We observed that the I6.45R mutant exhibits an exceptional pharmacological profile as it no longer gives a functional response when stimulated with the endogenous ligand histamine, but only responds to a particular class of synthetic H1R inverse agonists that contain tricyclic moieties such as doxepin and ketotifen. Mepyramine, an inverse agonist belonging to the class of biaryl H1R inverse agonists, is also unable to bind to the I6.45R mutant, suggesting that these two classes of H1R inverse agonists may bind the H1R in a different manner.