Signal transduction of the histamine H3 receptor

Bongers, G.M.

2008

Chapter 8

Discussion and conclusions

Discussion

The research in this thesis describes the pharmacological characterization and novel signal transduction of the human histamine H_3 receptor (hH_3R) and its isoforms. In short, we characterized the constitutive activity of the hH_3R, investigated the pharmacological difference between two H_3R isoforms and studied the G-protein coupling specificity of four hH_3R isoforms. Additionally we describe characterization two signaling routes of the hH_3R, namely activation of the heretofore unknown hH_3R-mediated activation of the Akt/GSK-3β axis and characterization of the hH_3R-mediated release of intracellular calcium ($[Ca^{2+}]_i$).

Constitutive activity of the H_3R

The cloning of the hH_3R in 1999 by Lovenberg et al. and opened up to the possibility to recombinantly express the hH_3R in cell lines and thereby facilitated its pharmacological characterization (Lovenberg et al., 1999). Like many other G-protein coupled receptors (GPCRs) (Costa and Cotecchia, 2005; Bond and Ijzerman, 2006), both the histamine H_1 receptor and histamine H_2 receptor, were shown to be constitutively activity upon recombinant expression in cell lines (Alewijnse et al., 1998; Bakker et al., 2000). Establishing constitutive activity of a G-protein coupled receptor (GPCR) is of importance, because the level of constitutive activity determines if antagonists would behave as inverse agonists or neutral antagonists. Although the level of constitutive activity greatly depends on its expression and cellular context, pharmacological characterization of ligands enables one to rank the inverse agonists based potency and efficacy. Furthermore, some compounds are known to behave as neutral antagonists and these potentially have the additional benefit over inverse agonists as they are less likely to up-regulate the expression of constitutively active GPCRs as was shown for the histamine H_1, histamine H_2 and serotonin 5-HT_{2C} receptor. This up-regulation is accompanied by an increased responsiveness to agonists even at low expression of the receptor (Smit et al., 1996; Bakker et al., 2000; Devlin et al., 2004). These data indicate that even when constitutive activity is not be detected *in vivo*, treatment with an inverse agonists might still alter receptor expression and thereby change the cellular physiology and most notably the responsiveness to the endogenous agonists (Sadee et al., 2005; Salamone et al., 2007).

Stable expression of the hH_3R in SK-N-MC cells, a neuroblastoma cell line, at moderate expression levels (~300 fmol/mg protein) revealed that indeed, like the histamine H_1, H_2 and H_4 receptor, the hH_3R is constitutively active (Chapter 3, 6). In these cells we show that the hH_3R signals in a constitutively active manner towards

the AC pathway (Chapter 3) and the Akt/GSK-3β pathway (Chapter 6). Additionally many classical H_3R antagonists (e.g. thioperamide, clobenpropit, ciproxyfan) were shown to reverse this constitutive inhibition of AC in these SK-N-MC cells, as well as in stably transfected CHO (Morisset et al., 2000) and HEK293 (Wulff et al., 2002) cells and are in fact H_3R inverse agonists. Moreover, we found that VUF4904 acts as neutral antagonist in the AC signal transduction pathway (Chapter 3). More recently N-isopropylimpentamine and a propylene analogue of immepip, VUF5681, was shown to be a neutral antagonists for this pathway as well. These neutral H_3R antagonists competitively blocked the effect of H_3R agonists and H_3R inverse agonists (Kitbunnadaj et al., 2003). Moreover, VUF5681 was shown to block the effects of H_3R inverse agonist thioperamide on *in vivo* protein kinase A-mediated synthesis of histamine in rat brain cortex, whereas it did not modulate histamine synthesis (Moreno-Delgado et al., 2006b). Both these H_3R neutral antagonists have only small chemical differences compared to a H_3R agonist of inverse agonists. In Chapter 3 we show that small differences between H_3R ligands can bestow a compound to be either a agonists, neutral antagonist or inverse agonist. Neutral antagonists might be of particular importance for the H_3R as it was one of the few GPCRs for which constitutive activity has been shown to occur *in vivo* (Morisset et al., 2000; Adan and Kas, 2003). Therefore, it has been suggested that for the H_3R neutral antagonist would be preferred over inverse agonists in the treatment of cognitive disorders (Schwartz et al., 2003).

Besides stable expression of the hH_3R in SK-N-MC cells, we also stably expressed the hH_3R in C6 cells (rat glioma cell line, Chapter 4) and transiently in COS-7 cells (African green monkey kidney fibroblast cell line, Chapter 5). Also in these cell lines the hH_3R displayed constitutive activity. More importantly, both in the COS-7 cells and in the C6 cells we characterized the constitutive activity of some of the hH_3R isoforms and from these results it becomes clear that notably one splice variant of the hH_3R receptor is highly constitutively active, namely the $hH_3R(365)$. In Chapter 4 we describe that this constitutive activity of the $hH_3R(365)$ affects the hH_3R pharmacology and inverse agonists are less potent and have a lower affinity for this splice variant as can be predicted from the cubic ternary complex model for a GPCR with an increased constitutive activity. This is likely is of great importance *in vivo* as well because the $hH_3R(365)$ and the $hH_3R(445)$ are both highly expressed in the CNS, at least as shown in Chapter 4 by RT-PCR and by others (Cogé et al., 2001; Wellendorph et al., 2002). One might even speculate that the high constitutive activity of this particular splice variant is reason why *in vivo* constitutive activity of the H_3R could be shown (Morisset et al., 2000). In Chapter 4 we described the H_3R-mediated constitutive activity in a very direct manner by [^{35}S]GTPγS binding and on the second messenger level, namely the inhibition of AC by measurement of cAMP in a protein kinase A binding assay. Unpublished

observations show that in these C6 cells an increase in constitutive activity for the $hH_3R(365)$ occurs in phosphorylation of ERK1/2 as well and H_3R-mediated activation of the Akt/GSK-3β axis. In Chapter 5 we show the constitutive activity by transient expression of the hH_3R in COS-7 cells and use a downstream signaling event, namely activity of cAMP-Responsive Element (CRE) that in turn drives the expression of a luciferase reporter gene facilitating the read-out. Moreover, we also show that the G-protein, upon equal expression, does not affect the relative constitutive activity of the tested hH_3R isoforms. For the higly constitutively active $hH_3R(365)$, the level of spontanous activity is not determined by the expressed G-protein. The reason why the $hH_3R(365)$ shows this enhanced constitutive activity remains elusive, however the $hH_3R(329)$ nor the $hH_3R(415)$ show this increase in constitutive activity. Alignment of these hH_3R isoforms indicates that the C-terminal region of the IL3 might be important in this increased constitutive activity. This region has been implicated for a number of GPCR in the modulation of constitutive activity (Seifert and Wenzel-Seifert, 2002; Pauwels et al., 2003) and is generally attributed to in increased coupling to the G-proteins (Johnston and Siderovski, 2007a; Johnston and Siderovski, 2007b) or might be related to movement of the bottom of transmembrane domain VI in response to the binding of agonist ligands (Hubbell et al., 2003). Additionally, this region has been shown to be important for scaffolding protein spinophilin, providing an alternative explanation for the observed increase of constitutive activity in this splice variant of the hH_3R. Spinophilin was shown to recruit regulators of G-protein signaling (RGS)-proteins to the third intracellular loop of GPCRs (Wang et al., 2005). Sufficient amounts of RGS proteins have been shown to increases the fraction of basal GTPase activity contributed by the constitutive activity of a GPCR and to modulated the efficacy of inverse agonists (Hoffmann et al., 2001; Welsby et al., 2002). In intact cells, RGS proteins and possibly other modulators of G protein function are expected to regulate the constitutive activity of GPCRs and thus the likely therapeutic effectiveness of inverse agonists (Milligan, 2003).

hH_3R splice variants

The cloning of the hH_3R revealed that the full length receptor is GPCR 445 amino acids long (Lovenberg et al., 1999). Subsequently several papers reported on the discovery of several hH_3R isoforms and in two of these paerps the expression of the hH_3R isoforms was determined by either Northern blot analysis or RT-PCR. We determined the expression of the $hH_3R(445)$ and $hH_3R(365)$ by RT-PCR in Chapter 4 and confirmed earlier repots of high expression of the $hH_3R(445)$ and $hH_3R(365)$ in the cerebellum, caudate, hypothalamus, cerebrum and the thalamus (Cogé et al., 2001; Wellendorph et al., 2002). However, all these techniques detect mRNA

levels which do not always reflect actual protein levels. As long as no specific radioligands are developed, which will be a difficult task at any rate, the actual expression of the hH_3R isoforms awaits the confirmation by specific antibodies raised against the different hH_3R isoforms.

To date only two papers described the pharmacological characterization of some of these hH_3R isoforms. The paper by Cogé et al. describes the cloning of six hH_3R isoforms and the characterization of three of them, the $hH_3R(445)$, $hH_3R(431)$ and $hH_3R(365)$. The $hH_3R(431)$ could not be detected by radioligand binding, whereas both the $hH_3R(445)$ and $hH_3R(365)$ showed [^{125}I]Iodoproxyfan radioligand binding. The lack of radioligand binding for the $hH_3R(431)$ can likely be attributed to the deletion of 14 amino acids in second transmembrane domain that eliminates a structurally important proline residue characteristic for amine receptors (Ballesteros et al., 2001; Visiers et al., 2002). [^{125}I]Iodoproxyfan competition binding experiments revealed a similar pharmacology for the $hH_3R(445)$ and $hH_3R(365)$, however the $hH_3R(365)$ was unable to elicit a functional response in response to H_3R agonists, neither for the inhibition of AC, release of intracellular Ca^{2+}, nor in a [^{35}S]GTPγS binding assay. The second report on the pharmacological characterization of the hH_3R isoforms by Wellendorph et al. describes the characterization of six hH_3R isoforms, the $hH_3R(445)$, $hH_3R(373)$, $hH_3R(365)$, $hH_3R(309)$, $hH_3R(301)$ and the $hH_3R(220)$. From these only the $hH_3R(445)$, $hH_3R(365)$ and $hH_3R(373)$ show N^{α}-[methyl-^{3}H]-histamine radioligand binding and a functional response to H_3R compounds as measure in a R-SAT™ reporter assay. The $hH_3R(309)$, $hH_3R(301)$ and the $hH_3R(220)$ lack many important residues important for function and the overall structural organization of a GPCR that is hardly is surprising that these are non-functional (Kristiansen, 2004). However, as was shown for the non-functional rat H_3R isoforms lacking TM7 we cannot excluded an indirect functional role by modulation the activity of the functional hH_3R isoforms by acting in a dominant active manner (Bakker et al., 2006). As described in contrast to the paper of Cogé et al., the paper of Wellendorph et al. shows a functional response of the $hH_3R(365)$ in response to stimulation with a H_3R agonist. The discrepancy between these papers formed basis to perform a detailed pharmacological characterization of the highly expressed $hH_3R(445)$ and $hH_3R(365)$ isoforms and is described in Chapter 4. Our data are in agreement with the paper of Wellendorp et al. with regard to the fact that the $hH_3R(365)$ is able to elicit a response after stimulation with a H_3R agonist. However, as shown in Chapter 4, the response of the $hH_3R(365)$ is much lower than the $hH_3R(445)$. Like Wellendorp et al. we indeed find that agonists are much more potent at the $hH_3R(365)$. In addition we also tested a panel of inverse agonists and found that these were less potent at the $hH_3R(365)$. The decrease in potency for inverse agonists and increased potency for agonists agrees with the proposal set forward

in Chapter 4 that the $hH_3R(365)$ is a constitutively active splice variant of the hH_3R. This is corroborated by the fact that the basal activity of the $hH_3R(365)$ is increased compared to the $hH_3R(445)$ for both the inhibition of AC and in a [^{35}S]GTPγS binding assay. Furthermore, we show that this is not due to an increase in expression of the $hH_3R(365)$ as determined by radioligand binding with agonists N^{α}-[methyl-^{3}H]-histamine and the inverse agonist [^{3}H]A-349821. These finding might also have clinical applications because although H_3R inverse agonist are more potent at the $hH_3R(445)$, at sufficient concentrations the inverse agonists are much more efficacious in inhibiting the constitutive activity of the $hH_3R(365)$. However, currently it is not know if the constitutive activity of the $hH_3R(365)$ is a determinant in the overall H_3R pharmacology or whether it plays a major role *in vivo*. It might be interesting to pursue this question by generation of transgenic mice expressing either the $H_3R(445)$ or the $H_3R(365)$ conditionally in a $H_3R^{-/-}$ background to address questions of constitutive activity of the H_3R during development and with regard the responsiveness of the H_3R towards H_3R inverse agonists.

Many splice variant have been described for the hH_3R, with deletions in the N-terminal part, IL3 and even lacking complete TM-domains. Potentially the most interesting splice variants are the ones that have variations in the third intracellular loop, as this region has been implicated in the binding to various proteins (Abramow-Newerly et al., 2006b). One family of proteins associated with the third intracellular loop are the G-proteins, which have been the subject of investigation in Chapter 5 of this thesis. Although for some GPCRs altered pharmacology has been observed for isoforms that have deletions in the IL3, we did not see major changes in the agonist mediated pharmacology upon expression of the tested PTX-insensitive $G\alpha_{i/o}$-proteins However, its seems that pharmacology of the potency of H_3R inverse agonists depends on the expression of the $G\alpha_{i/o}$-proteins. At the moment no clear explanation is available for this observation. However, not much is know at the moment of the interaction with other proteins. Important effector proteins, like β-arrestin, spinophilin and possibly RGS proteins, are known to interact with the third intracellular loop. All these proteins might effect the signaling of the other by a direct competition for binding of an influencing the signaling of the other, as described for modulation of G-proteins GTPase activity by RGS proteins by either enhancing the GTPase activity via their regulators of G protein signaling domain or inhibiting it through their guanine nucleotide dissociation inhibitor domain (Mittal and Linder, 2004; Shu et al., 2007).

The β-arrestin mediated signal transduction of GPCRs has been shown to be important in the down-regulation of signaling and in the activation ERK1/2. The activation rat H_3R has been shown to lead to ERK1/2 phosphorylation and

unpublished observation have shown this to be the case for the hH_3R as well. To date nothing is known about the role of β-arrestin is this important signal transduction event. Additionally there might be differential regulation of β-arrestin mediated activation ERK1/2 by the different hH_3R isoforms as well as the potential difference in β-arrestin mediated down-regulation of hH_3R signaling, features that so far have not been investigated.

Besides the hH_3R isoforms with variations in the third intracellular loop other hH_3R isoforms have been described with modifications at the N-terminus, isoforms $hH_3R(409)$, $hH_3R(395)$, $hH_3R(379)$, $hH_3R(329b)$, $hH_3R(293)$ and $hH_3R(290)$. All these isoforms lack 36 amino acids at the N-terminus containing a N-glycosylation site. These isoforms are thus far not characterized and might be interesting to study as glycosylation at GPCRs has been found to be important in the stabilization dimers (Michineau et al., 2006) and for correct trafficking to the membrane (Rands et al., 1990; Servant et al., 1996; Lanctot et al., 2005). Additionally, the third intracellular loop is suggested by some to play a major role in the formation of dimers through the formation of coiled-coil structures and potentially complicates the hH_3R pharmacology of the hH_3R isoforms that have deletions in the third intracellular loop even more. In general not much is know about the dimerization of the hH_3R, but especially regarding the multitude of naturally occurring isoforms with deletions in the third intracellular loop provides an important tool to test the importance of regions in this loop and its role in dimerization.

As described the potential differential role of the hH_3R isoforms in signaling remains largely elusive and even more fundamentally nothing is know about regulation of the expression of the hH_3R isoforms. The $hH_3R(445)$ is shown to be highly up-regulated during developments suggesting it plays an important role during development (Karlstedt et al., 2003). It would be highly interesting to know whether there is a specific role for some of these hH_3R isoforms and how the pattern of hH_3R expression is regulated.

To study the role of these hH_3R isoforms it might be interesting to knock-in one of the hH_3R isoforms in a H_3R knock-out background. In mice however, the multitude of hH_3R isoforms is not found so far, a observation that might reflect the higher order of complexity in humans with respect to proteins being able to bind to the hH_3R needing differential signaling GPCRs. Studying these events in a more human setting is more difficult and as far a know no hH_3R isoform specific compounds are currently available.

hH_3R mediated signaling

Before the cloning of the hH_3R, its was suggested that the hH_3R might couple to the pertussis toxin (PTX) sensitive class of $G\alpha_{i/o}$-proteins (Clark et al., 1993; Endou

et al., 1994; Clark and Hill, 1995; Clark and Hill, 1996). Expression in cell lines after the cloning of the hH_3R confirmed its coupling to $G\alpha_{i/o}$-proteins and subsequently revealed at least three signaling pathways for the H_3R: $G\alpha_{i/o}$-mediated inhibition of AC (Lovenberg et al., 1999; Morisset et al., 2000; Cogé et al., 2001; Wieland et al., 2001; Gomez-Ramirez et al., 2002; Uveges et al., 2002; Sanchez-Lemus and Arias-Montano, 2004), stimulation of p44/p42 mitogen activated protein kinase (MAPK) pathway (Drutel et al., 2001) and coupling to neuronal Na^+/H^+ exchange (Silver et al., 2001). For some of these pathways the relevance in the H_3R mediated physiology still has to be established.

For the H_3R-mediated modulation of AC its and the subsequent change in cAMP levels has been shown to play a role in the synthesis of histamine and the exocytosis of of norepinephrine (Gomez-Ramirez et al., 2002; Seyedi et al., 2005; Torrent et al., 2005; Moreno-Delgado et al., 2006b). However, some studies have shown the inhibition of cholinergic neurotransmission in the guinea pig ileum and the release of norepinephrine from mouse cortex to be independent of AC (Poli et al., 1993; Schlicker et al., 1994a; Lee and Parsons, 2000). A less explored part of the AC pathway is the role of the cAMP binding-protein protein kinase A. Protein kinase A is know to play an important role in a range of biological responses including gene expression, synaptic plasticity, and behavior (Brandon et al., 1997).

In Chapter 6 we describe a novel hH_3R-mediated signal transduction, the activation of the Akt/GSK-3β axis, and investigated the hH_3R mediated release of intracellular calcium. We show that activation of the hH_3R in a neuroblastoma cell line, primary cultures of cortical neurons and in striatal slices of Spraque-Dawley rats activates the Akt/GSK-3β axis in a phospho-inositol-3-kinase dependent manner. Moreover, at least in a neuroblastoma cell line this occurs in a constitutively active manner as well. The exact role of this pathway in the H_3R-mediated physiology remains to be determined. In view of the predominantly CNS expression of the hH_3R and the fact that the Akt/GSK-3β axis is known to play a prominent role in brain function one might speculate this H_3R-mediated pathway to play an important role in neuronal migration or protection against neuronal apoptosis (Brazil et al., 2004). With respect to protection against apoptosis some papers claim that the H_3R might play a neuroprotective role in the CNS (Adachi et al., 1993). Additionally, H_3R mRNA is up-regulated in certain brain areas after induction of ischemia and kainic acid-induced seizures (Lintunen et al., 2005; Lozada et al., 2005). An interesting aspect not explored regarding the Akt/GSK-3β signaling is the described mediation of neurite outgrowth. Regarding the almost exclusive expression of the hH_3R in the central nervous system, its role on memory formation and its potential role in development, the investigation of the effect of the hH_3R on neurite outgrowth might be an interesting phenomenon to pursue.

Additionally, the Akt/GSK-3_ axis is believed to be altered in Alzheimer's disease, neurological disorders and schizophrenia (Li et al., 2002; Emamian et al., 2004; Rickle et al., 2004). Clearly future research needs to be done to evaluate the merit of these signaling events *in vivo*. The c-Fos mediated signaling has been shown to be important for the hH_3R pharmacology of the different pharmacological classes of H_3R ligands in being active in models of obesity or memory (Hancock et al., 2006). It would be interesting to see if these ligands also discriminate between the H_3R-mediated signaling pathways know to be involved in memory process, the Akt/GSK-3_ and the ERK1/2 signaling.

In Chapter 7 we show that activation of the hH_3R stably expressed in SK-N-MC cells results in a rapid and transient $G\alpha_{i/o}$-protein dependent calcium mobilization from intracellular stores. Furthermore, we show that this hH_3R-mediated release of intracellular calcium is independent of extracellular calcium, does not involve ryanodine receptors and most likely originates from inositol 1,4,5-trisphosphate receptor sensitive stores in a phospholipase C-dependent manner. For the related $G\alpha_{i/o}$-coupled histamine H_4 receptor a similar to calcium mobilization is shown in mast cells and eosinophils (Raible et al., 1994; Hofstra et al., 2003). In contrast, there are reports that activation of the H_3R reduces the K^+-induced intracellular calcium mobilization in SH-SY5Y cells. This phenomenon was linked to inhibitory effect of the H_3R on the norepinephrine exocytosis in these cells as well as in cardiac synaptosomes (Silver et al., 2002). Subsequently this H_3R-mediated inhibition of K^+-induced calcium mobilization was shown to be dependent on the H_3R-mediated inhibition of protein kinase A activity. Inhibition of proteins kinase leads to \decreased Ca^{2+} influx through voltage-operated Ca^{2+} channels (Seyedi et al., 2005). In contrast to the results described in Chapter 7 no effects on the intracellular Ca^{2+} levels were observed upon H_3R activation before the K^+-induced calcium release. Clearly more work has to be done to delineate the differences between these cells, or actually what is specific to these SK-N-MC cells, that can explain these contrasting observations. Additionally, it would be interesting to see whether our finding described in Chapter 7 can be seen in certain cell types *in vivo*.

With respect to non G-protein mediated signaling we developed and characterized a G-protein uncoupled mutant hH_3R that is able to bind ligands but does not signal to CRE anymore. This $R^{3.50}A$ mutants has been described for many other GPCR to uncouple the receptor of the G-proteins (Acharya and Karnik, 1996; Shibata et al., 1996; Lu et al., 1997; Scheer et al., 1997; Alewijnse et al., 2000; Scheer et al., 2000; Chung et al., 2002) and might be a valuable tool to investigate the heretofore unknown role of hH_3R mediated G-protein independent signaling. This residue is part of the conserved (D/E)RY motif C-terminal end of TM3 and have been shown to play an important roles in signaling for a large number of receptors (Flanagan, 2005; Rovati et al., 2007). The $R^{3.50}$ is one of the most conserved residues in

rhodopsin-like GPCRs R3.50 and is thought to be important in the formation of intramolecular interactions that constrain the receptor in either an inactive or activate conformation and mutation of this residue has been shown to impair G-protein coupling (Flanagan, 2005).

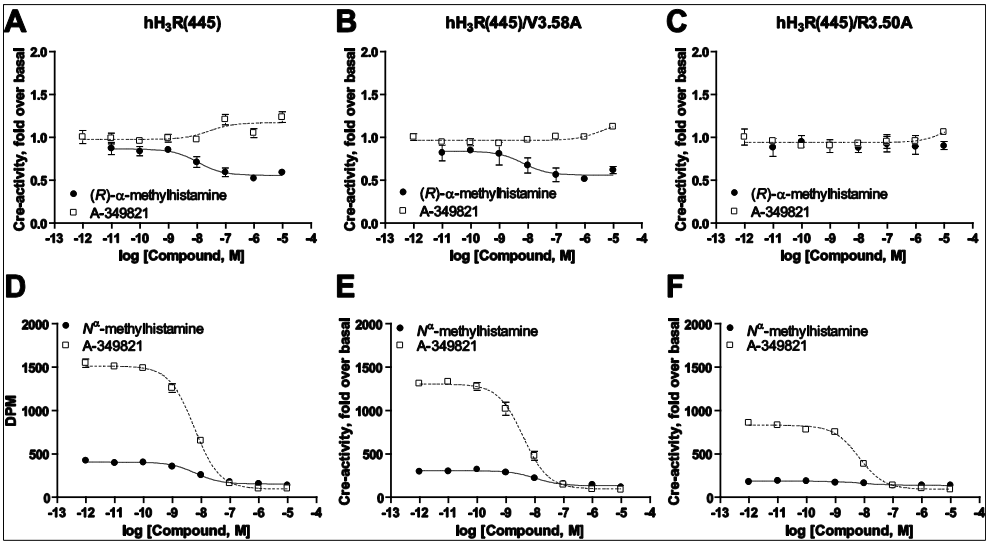

Figure 16. Uncoupling the hH_3R. Point mutation of $R^{3.50}A$ (C), but not $V^{3.58}A$ (B), prevents the H_3R-mediated modulation of CRE compared to wild-type (A), whereas, radioligand binding of both the agonists N^{α}-methylhistamine and the inverse agonists A-349821 is not markedly affected. These results suggest, as was shown for other GPCRs (for references, see text), that $R^{3.50}A$ mutation prevetents the H_3R from interacting with G-proteins.

Concluding remarks

Two members of the histamine receptor family, the histamine H_1 receptor and histamine H_2 receptor have proven to be a very lucrative drug target to develop specific ligands. The two remaining know histamine receptors, the H_3R and histamine H_4 receptor, still have to prove their merit. Especially for the H_3R a lot of pre-clinical evidence has recently been generating supporting the H_3R as an attractive drug target.

The H_3R has been pharmacologically almost xx years ago, but the cloning of the hH_3R has really pushed the field forward with the subsequent dedication of the pharmaceutical industry as well as ongoing efforts of academia. Clinical models of the hh_3R show that the compounds targeting the hH_3R might prove beneficial in several disease areas. However, although clinical trails are ongoing, to date nothing is known about its effect in human disease. To really prove the hH_3R as

being an attractive drug target the field is anxiously awaiting the outcome of these clinical trails.

The cloning of the hH_3R has led to the discovery of several signal transduction pathways that are modulated by the hH_3R. Some of these signaling pathways can be linked to relevant (patho)physiologies, like the hH_3R-mediated inhibition of the NHE. H_3R-mediated inhibition of NHE leads to a subsequent lowering in the exocytosis of norepinephrine and explaining the protective role of H_3R agonists during myocardial ischemia. For others like the Akt/GSK-3_ axis described in this thesis future research will have to prove the exact role of this signaling event in the H_3R-mediated physiology.

During the time of this thesis the hH_3R has become and increasingly interesting drug-target as a consequence of the increase of *in vivo* data that became available on the efficacy of hH_3R compounds in clinical models. On the other hand, matters became more complicated with the discovery of over 20 hH_3R isoforms for which the individual roles remain elusive. Still, although the molecular aspect of the H_3R might have become more complex, the H_3R remains an attractive drug target. This claim is substantiated by the recent progress to clinical phase studies. The field is waiting for the outcome of these studies to once and for all show the therapeutic potential of H_3R inverse agonists and possibly even H_3R agonist.