The Role of Transmembrane Helix 5 in Agonist Binding to the Human H3 Receptor

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ABSTRACT

We have used alanine scanning mutagenesis to identify residues in transmembrane domain 5 of the histamine H3 receptor that are important for agonist binding. All of the mutants generated were functionally expressed as demonstrated by their ability to bind [3H]iodoproxyfan with comparable affinity to the wild-type receptor and their ability to inhibit forskolin-stimulated cAMP formation when activated by histamine. Mutations produced small changes in the potency of histamine, but the most pronounced reduction in potency and affinity of the agonists, histamine, R-α-methylhistamine, imetit, and impentamine, was seen with mutation of glutamate 206. Our modeling suggests that this residue plays a key role in ligand binding by interacting with the imidazole ring of histamine. Interestingly, L199A greatly reduced agonist potency in functional assays but had only minor effects on agonist affinity, implicating a role for this residue in the mechanism of receptor activation. We also studied the functional effects of the mutations by linking the receptor to calcium signaling using a chimeric G protein. A comparison of the two functional assays demonstrated contrasting effects on agonist activity. Histamine, imetit, and impentamine were full agonists in the cAMP assay, but imetit exhibited only partial agonist activity through the chimeric G protein. Furthermore, impentamine, another potent agonist in the cAMP assay, was only able to activate the E206A mutant in the calcium assay despite being inactive at the wild-type receptor. These observations suggest that the agonist receptor complexes formed by these three different H3 agonists are not conformationally equivalent.

The neurotransmitter histamine is important in many diverse physiological mechanisms, and these actions are mediated through four distinct G protein-coupled receptors (GPCRs), which differ in their distribution, pharmacology, and function (Hill et al., 1997). Thus, the human H1 receptor, which is important in the allergic response, couples to phospholipase C activation when expressed in Chinese hamster ovary cells (Fukui et al., 1994). The H2 receptor couples to the stimulation of adenyl cyclase (Gantz et al., 1991) and has important functions in the control of gastric acid secretion. The cloned human H3 receptor subtype couples to Gai and the inhibition of cAMP production (Lovenberg et al., 1999). It has key modulatory roles in the release of neurotransmitters including histamine itself, γ-aminobutyric acid, noradrenaline, and acetylcholine and has been implicated in arousal and cognition. The recently cloned H4 receptor is also coupled to the inhibition of cAMP formation although, as yet, little is known about its function in vivo (Nakamura et al., 2000; Oda et al., 2000; Liu et al., 2001; Nguyen et al., 2001; Zhu et al., 2001).

The histamine receptors are members of the biogenic amine receptor subfamily of GPCRs. The ligand binding site in these receptors lies within a pocket formed by the seven transmembrane (TM) domains. Across the superfamily of GPCRs, there exist many residues that have been conserved throughout evolution and are thus thought to play key roles in receptor structure and/or function. Site-directed mutagenesis has demonstrated the importance of many of these residues in a number of different biogenic amine receptors, including the histamine H1 and H2 receptors. For example, mutation of the conserved TM3 aspartate has a profound effect on binding the positively charged biogenic amine ligands (Fraser et al., 1989; Gantz et al., 1992; Page et al., 1995). This has been further supported by the observation that the mustard ligands irreversibly alkylate this residue in rat brain M1 muscarinic receptors (Curtis et al., 1989; Spalding et al., 1994).

The interaction of the ligand with the TM3 aspartate facilitates binding to residues in other TM domains, particularly TMs 4 through 7. The critical role of TM5 has been demonstrated in many receptors including the β2- and α2A-adrenergic receptors (Strader et al., 1989; Wang et al., 1991), the M3 muscarinic receptor (Wess et al., 1992), and dopamine D1 receptor (Pollock et al., 1992). Furthermore, histamine is also thought to interact with TM5 residues in both the H1 and H2 receptors. Leurs et al. (1994) demonstrated...
the importance of threonine 203 in the binding of histamine to the H1 receptors and suggested that it interacts with the imidazole ring, because a nonimidazole H1 receptor agonist is less sensitive to its substitution to alanine. Throneine 190 in the H2 receptor plays a similar role in ligand binding (Gantz et al., 1992). More recently, a modeling and mutational study identified, in the H1 receptor, lysine 200 as another TM5 residue that is important for ligand binding (Wieland et al., 1999).

Sequence alignments show that TM5 of the histamine receptors is poorly conserved (Fig. 1), suggesting a potential difference in the mechanism in which histamine binds to the H3 receptor. To investigate the interaction of histaminergic ligands with TM5 of the H3 receptor, we have performed an alanine scan of the first 14 amino acids and investigated their effect using radioligand binding, cAMP assays, and the fluorometric imaging plate reader (FLIPR), in which receptor activation is coupled to calcium mobilization. A preliminary account of this work has been presented previously (Uveges and Jones, 2001). In short, our results suggest that residues in TM5 play key roles in agonist-induced activation of the H3 receptor, and interestingly, these amino acids align with key residues identified in the other histamine receptors. Furthermore, our data suggest that the agonist receptor complexes formed by three full agonists, histamine, imetit, and impentamine, are not equivalent and provide a new insight into H3 pharmacology.

**Materials and Methods**

Cell Line Generation. The human histamine 3 receptor cDNA was cloned from a thalamus library and subcloned into the pCDNA3.1+ zeo vector (Invitrogen, Carlsbad, CA). HEK Tsa cells were transfected, and stable clones were selected with 500 μg/ml zeocin. Clones expressing wild-type or mutant H3 receptors were identified by reverse transcription-polymerase chain reaction and pharmacological analysis.

In Vitro Mutagenesis. Mutagenesis of the human H3 receptor was performed using the QuikChange mutagenesis kit (Stratagene, La Jolla, CA). The mutation and full-length sequence were confirmed by sequencing on an ABI 3700 (ABI, Foster City, CA) capillary DNA sequencer, the open reading frame was subcloned into the mammalian expression vector pCDNA3.1+ zeo (Invitrogen), and stable lines were prepared.

Iodoproxyfan Binding. [125I]Iodoproxyfan (Amersham Biosciences, Piscataway, NJ) was incubated with cell membranes and prepared from the stable line at 30°C for 1 h in 50 mM Tris-HCl, pH 7.4. Twenty-five pM [125I]iodoproxyfan was used for the competition assays. They were filtered on Whatman GF/B paper (Whatman, Clifton, NJ) presoaked in 0.3% polyethyleneimine, washed with ice-cold water, and radioactivity was determined in a Packard beta counter (Packard, Downers Grove, IL). Nonspecific binding was determined using 1 μM histamine or R-α-methylhistamine (1 mM ligand for E206A). Impentamine was synthesized in-house, and all other chemicals were purchased from RBI/Sigma (St. Louis, MO). IC₅₀ values were converted to Kᵢ values using the Cheng-Prusoff equation.

Adenylyl Cyclase Assays. Cells were plated into 96-well Biocoat culture plates (BD Biosciences, San Jose, CA) 24 h prior to assay. They were incubated in 100 μl of Krebs-bicarbonate buffer at 37°C for 15 min, followed by a 5-min incubation in the presence of 0.5 mM isobutylmethyl xanthine. The cells were then stimulated for 12 min with agonist in the presence of 10 μM forskolin. The reaction was terminated by the addition of 20 μl of 0.5 M perchloric acid, and the cAMP levels were determined using the scintillation proximity assay (Amersham Biosciences). EC₅₀ values were calculated using GraphPad Prism (GraphPad Software Inc., San Diego, CA).

Ca²⁺ Imaging Studies. H3 receptor coupling to increases in intracellular free calcium was achieved by coexpression of the H3 receptor with a chimeric Gqα, in which the last five C-terminal amino acids were replaced with those from Gαi3. Wild-type H3 receptor or mutants were transiently coexpressed with the G protein chimera in HEK-293 cells, and agonist-stimulated calcium mobilization was evaluated using the FLIPR (Molecular Devices, Menlo Park, CA). Cells plated at 50,000/well in 96-well plates were loaded with the calcium indicator dye Fluo-3 in Hanks’ buffered saline solution for 60 min at 37°C. Cells were washed with Hanks’ buffered saline solution at room temperature and transferred to FLIPR for acquisition of calcium images. Images were captured at 1-s intervals, and cells were stimulated by addition of agonist 20 s after the beginning of data collection. For each mutant, data were normalized to the change in fluorescence observed with a maximally effective concentration of histamine.

Molecular Modeling. The crystallographically determined coordinates for bovine rhodopsin (Palczewski et al., 2000) were retrieved from the Protein Data Bank. All computations were performed using the Molecular Operating Environment. The structure was read into the program along with the sequence of the human H3 receptor subtype. Sequence alignment was performed using the pam250 algorithm, followed by manual adjustment to avoid insertions or loops in defined secondary structural domains. Structure optimization was performed using the Molecular Operating Environment implementation of the Kollman force field, employing 100 steps of steepest descent, followed by 100 steps of conjugate gradient, and finally, 200 steps of truncated Newton-Raphson. A model of histamine in the protonated form was “locked” into the transmembrane helical domain of the receptor structure following removal of the intra- and extracellular loops. Helix 5 was manually manipulated such that glutamate 206 was able to form an intermolecular interaction with the imidazole ring of the histamine ligand. The ligand-receptor complex was further optimized using the Merck molecular force field under the conditions previously employed.

**Results**

Effects of Mutations on Agonist-Induced Inhibition of Forskolin-Stimulated cAMP Accumulation. The ability of histamine to inhibit forskolin-stimulated cAMP formation at the wild-type and mutant receptors is summarized in Table 1, and representative dose response curves are presented for the wild type and seven mutants in Fig. 2. Many of the mutations had only minor effects on potency (Table 1). Small 2- to 5-fold increases in potency were observed in several mutants, most notably, tryptophan 196 and T204A. Others reduced histamine’s potency by 4- to 22-fold, with the largest effects being demonstrated for L199A, A202Q, and E206A and by mutation of the highly conserved phenylalanine 207. Furthermore, the L199A, I200A, A202Q, and E206A mutations led to small decreases in efficacy. There was no evidence for constitutive activity of any of the receptors studied. Figure 3 shows the effects of four full agonists, histamine, R-α-methylhistamine, imetit, and impentamine, on the forskolin-induced cAMP levels in cells expressing the wild-type.
TABLE 1
Effects of TM5 mutations on the inhibition of forskolin-induced cAMP levels
HEK 293 cells expressing wild-type or mutant H3 receptors were plated in 96-well plates 24 h prior to assay. The ability of histamine to inhibit 10 μM forskolin-induced cAMP levels was determined during a 12 min stimulation in the presence of 0.5 mM IBMX. cAMP levels were determined using the scintillation proximity assay, and the results shown are the mean ± S.E. of three independent experiments performed in triplicate.

<table>
<thead>
<tr>
<th></th>
<th>Histamine pIC50 (IC50 nM)</th>
<th>Maximal Percent Inhibition Forskolin-Induced cAMP Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>8.63 ± 0.10 (2.32)</td>
<td>91.6</td>
</tr>
<tr>
<td>W196A</td>
<td>9.30 ± 0.10 (0.50)</td>
<td>89.6</td>
</tr>
<tr>
<td>Y197A</td>
<td>9.03 ± 0.09 (0.93)</td>
<td>94.8</td>
</tr>
<tr>
<td>F198A</td>
<td>8.66 ± 0.11 (2.19)</td>
<td>83.8</td>
</tr>
<tr>
<td>L199A</td>
<td>7.45 ± 0.10 (35.3)</td>
<td>71.9</td>
</tr>
<tr>
<td>E206A</td>
<td>7.98 ± 0.17 (10.5)</td>
<td>76.9</td>
</tr>
<tr>
<td>T201A</td>
<td>8.96 ± 0.05 (1.11)</td>
<td>94.9</td>
</tr>
<tr>
<td>A202Q</td>
<td>7.39 ± 0.12 (40.80)</td>
<td>72.0</td>
</tr>
<tr>
<td>S203A</td>
<td>8.67 ± 0.04 (2.13)</td>
<td>84.5</td>
</tr>
<tr>
<td>T204A</td>
<td>9.19 ± 0.12 (0.64)</td>
<td>93.7</td>
</tr>
<tr>
<td>L205A</td>
<td>8.94 ± 0.05 (1.16)</td>
<td>95.0</td>
</tr>
<tr>
<td>E206A</td>
<td>7.29 ± 0.12 (51.48)</td>
<td>74.1</td>
</tr>
<tr>
<td>F207A</td>
<td>7.39 ± 0.16 (40.41)</td>
<td>86.7</td>
</tr>
<tr>
<td>F208A</td>
<td>7.86 ± 0.04 (13.69)</td>
<td>88.3</td>
</tr>
<tr>
<td>T209A</td>
<td>8.93 ± 0.01 (1.18)</td>
<td>93.2</td>
</tr>
</tbody>
</table>

Fig. 2. Histamine dose-response curves for wild-type and mutant H3 receptors. Stable cell lines were stimulated for 12 min in the presence of 10 μM forskolin and 0.5 mM isobutylmethylxanthine. Levels of cAMP were determined using the scintillation proximity assay technology. Representative curves, performed in triplicate, are shown. ■, wild type; ×, W196A; ▲, L199A; ○, T201A; ▼, A202Q; ◇, T204A; ●, E206A; □, F208A.

Role of TM5 in Agonist Binding to the Human H3 Receptor

The wild-type H3 receptor bound iodoproxyfan with a Kᵢ of 24.5 ± 7.5 pM. The Bₘₐₓ for the wild-type receptor was 400 pmol/mg of protein. The mutations caused little (less than 2-fold) effect on the Kᵢ of iodoproxyfan (data not shown). The Bₘₐₓ for the mutants ranged from 30 to 400 pmol/mg of protein. For the wild-type H3 receptor, a 20-fold difference in Bₘₐₓ did not appear to affect the affinity or potency of histamine (data not shown).

At the wild-type receptor, R-α-methylhistamine and imetit gave rise to displacement curves that fit to a two-site model marginally better than to a single site. Approximately 20% of the sites were higher affinity. Surprisingly, histamine itself did not reliably fit a multisite model. Impentamine fit to a one-site model at the wild type, and all ligands with the mutants gave single component curves, which is consistent with the lower potency and efficacy of the agonists at the mutated receptors. A lower Bₘₐₓ of some mutants may also have contributed to the inability to distinguish a higher affinity site.

The loss of the high-affinity state results in analysis of the low-affinity binding. Displacement curves indicated decreases in affinity of histamine for most mutants. Glutamate 206 showed a large, 2052-fold decrease in low affinity binding, where the Kᵢ was determined to be 32.5 μM. In agreement with the functional data, E206A had the greatest effect on the affinity of the agonists, with histamine and R-α-methylhistamine being affected the most. In the binding assay, imentamine was relatively unaffected by the mutations. L199A had little or only small effects on the binding affinity. The increases in potency seen with W196A and T204A in the functional data were not reflected in the binding to the low-affinity, uncoupled receptor.

Effects of the Mutations on Receptor Activity in the FLIPR Assay. The wild type and mutants were also studied using the FLIPR assay, in which the H3 receptor activation is coupled to calcium release via Goq/G11 chimera (Goq containing the five C-terminal amino acids of Gai). No calcium response was seen in the absence of the chimera (Fig. 4). The
results obtained, summarized in Table 4 and Fig. 5, are comparable with those obtained in the cAMP assay, although in the case of the wild-type receptor, the half-maximal concentrations of the agonists studied were increased 6- to 9-fold (15.4, 3.16, and 1.38 nM for histamine, methylhistamine, and imetit, respectively, compared with 2.32, 0.34, and 0.16 nM in the cAMP assay). The effects of the mutations were also exaggerated in this assay (e.g., E206A led to a 45-fold decrease in histamine potency compared with the 22-fold change seen in the cAMP assay). The mutation of glutamate 206 had the greatest effect on the agonists studied, particularly on \( R\)-\( \alpha \)-methylhistamine, for which \( EC_{50} \) was increased 2499-fold. The increase in affinity seen in the cAMP assay is supported by the data obtained in this assay. However, no response with any agonist was seen with the W106A mutant despite the 5-fold increase in potency seen in the cAMP assay.

Other notable differences between the two functional assays are the apparent partial agonist nature of imetit observed consistently across wild type and all mutants and the observation that impentamine, a potent full agonist in the wild-type H3 cAMP assay (Fig. 3), failed to stimulate the wild-type receptor in the FLIPR assay with the exception of E206A.

**Molecular Modeling.** To understand the molecular basis of the results described here, a model of histamine docked into the transmembrane domain of the H3 receptor was made (Fig. 6), based on the crystal structure of bovine rhodopsin. The basic nitrogen of the histamine molecule interacts with the conserved aspartate residue (aspartate 114) in TM3, which has been implicated in ligand binding. Additionally, the imidazole amino group forms an intermolecular hydrogen bond to the acid group of glutamate 206, consistent with our findings that this residue participates in ligand binding. The methyl side chain of alanine 202 buttresses against the aforementioned glutamate. Mutation of alanine 202 to the more bulky asparagine would be expected to change both the steric and electrostatic nature of this domain and thus adversely affect the affinity of ligand binding. The remainder of the
binding pocket is formed by hydrophobic aromatic residues: a tyrosine residue (tyrosine 115) adjacent to asparagine 114 in TM3 sits below the histamine, with tyrosine (tyrosine 374) in TM6 and phenylalanine (phenylalanine 394) residues from TM7 completing the pocket.

**Discussion**

The elucidation of the molecular mechanisms involved in receptor ligand interactions is important in the design of potent and selective ligands. Current opinion is that positively charged biogenic amine ligands, such as histamine, interact with a conserved TM3 asparate residue, permitting cooperative interaction with residues from multiple TM domains, especially TM5. Mutation of asparate 114 in the human H3 receptor to either asparagine or glutamate resulted in no detectable cAMP response or specific binding of the selective H3 antagonist \[^{125}\text{I}]\text{iodoproxyfan, even though the human H3 receptor to either asparagine or glutamate}

<table>
<thead>
<tr>
<th>Binding Ligand</th>
<th>Histamine pEC\text{50}</th>
<th>R-α-Methylhistamine pEC\text{50}</th>
<th>Imetit pEC\text{50}</th>
<th>Impentamine pEC\text{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>7.82 ± 0.09 (85%)</td>
<td>8.50 ± 0.13 (88%)</td>
<td>8.86 ± 0.20 (54%)</td>
<td>N.D.</td>
</tr>
<tr>
<td>L199A</td>
<td>6.22 ± 0.10 (108)</td>
<td>6.72 ± 0.11 (148)</td>
<td>7.04 ± 0.18 (71)</td>
<td>N.D.</td>
</tr>
<tr>
<td>T201A</td>
<td>8.41 ± 0.12 (104)</td>
<td>8.85 ± 0.11 (102)</td>
<td>9.06 ± 0.23 (61)</td>
<td>N.D.</td>
</tr>
<tr>
<td>A202Q</td>
<td>7.00 ± 0.08 (100)</td>
<td>7.93 ± 0.15 (98)</td>
<td>8.10 ± 0.20 (55)</td>
<td>N.D.</td>
</tr>
<tr>
<td>T204A</td>
<td>7.54 ± 0.16 (99)</td>
<td>8.56 ± 0.22 (108)</td>
<td>8.46 ± 0.23 (64)</td>
<td>N.D.</td>
</tr>
<tr>
<td>E206A</td>
<td>6.16 ± 0.09 (101)</td>
<td>5.10 ± 0.06 (95)</td>
<td>6.80 ± 0.14 (83)</td>
<td>7.46 ± 0.13 (70)</td>
</tr>
<tr>
<td>F208A</td>
<td>7.16 ± 0.08 (106)</td>
<td>8.63 ± 0.18 (108)</td>
<td>7.61 ± 0.33 (37)</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not detectable.

\[^{a}\] Values in parentheses represent percent maximal response.

aspartate 186 and threonine 190 in TM5 of the H2 receptor, although only minor differences in maximal responses were seen in the H1 or the present study. The role of threonine 190 in defining the affinity and efficacy of H2 ligands may be through a hydrogen bond interaction between its hydroxyl side chain and the imidazole ring of the ligand (Gantz et al., 1992). Aspartate 186 and threonine 190 align with H3 residues alanine 202 and glutamate 206, respectively. In this study, the potency of histamine at both the A202Q and E206A mutants was decreased approximately 20-fold, whereas the effects of mutating the glutamate 206 were much greater in the binding studies (2000-fold decrease in affinity). Similar effects were seen with other ligands, and this suggests that agonist binding to the uncoupled receptor, measured by the binding, is affected to a greater extent than the high-affinity state of the receptor through which the functional response is mediated.

Our molecular model shows an interaction of the imidazole ring of histamine with glutamate 206. It also indicates that the effects of the A202Q mutation may be indirect through steric influences of the bulkier glutamate residue on glutamate 206, explaining the smaller functional effects of the alanine 202 mutation. A glutamate appears at this position in both the H3 and H4 receptors, both of which bind histamine with a much higher affinity than either H1 or H2 receptors. This increased affinity may be due, in part, to a stronger interaction of histamine’s imidazole ring with the glutamate residue, compared with the asparagine and threonine found in the H1 and H2 receptors, respectively. The presence of the glutamate residue also coincides with the ability of the H3 and H4 receptors to bind the selective agonist R-α-methylhistamine with high affinity, and interestingly, R-α-methylhistamine is the most sensitive agonist to the E206A mutation.

Alanine 202 and glutamate 206 are also equivalent to amino acid residues that have been shown to play key roles in ligand binding in other biogenic amine receptors. For instance, glutamate 206 aligns with serine 207 in the β2-adrenergic receptor, which is one of two TM5 serine residues that have been shown to interact with the hydroxyls of the catechol ring of adrenaline (Strader et al., 1989). Alanine 202 aligns with serine 198 in the D1 receptor, which has been reported to be important for the binding of dopamine and other ligands (Pollock et al., 1992). Together, these observations demonstrate a functional conservation of TM5 across the GPCR family. However, care must be exercised in the interpretation of such results since our model does not sup-
Fig. 5. Dose-response curves for histamine, R-α-methylhistamine, imetit, and impentamine were determined using the FLIPR. The wild-type and mutant H3 receptors were coupled to calcium mobilization, and dose-response curves were prepared. Data presented represent data obtained in two independent experiments, each using triplicate determinations for all data points. The results are expressed as percentage of the maximum histamine response. ■, histamine; ▲, R-α-methylhistamine; ●, imetit; ♦, impentamine.
port a direct interaction of alanine 202 with histamine, and
furthermore, serine 200 in the α2A-adrenergic receptor, also
equivalent to alanine 202, appears not to be involved in
agonist binding (Wang et al., 1991). Different receptor-ligand
interactions may therefore use a different subset of TM5
residues.

Modeling and mutational studies (Leurs et al., 1995; ter
Laak et al., 1995) suggest that the lysine 200 in the H1
receptor is important for receptor activation. Mutation of
the corresponding H3 residue leucine 199 reduced the potency
and efficacy of agonists in the cAMP assay. Our modeling
proposes that leucine 199 is distant from the binding site,
suggesting that it may play a role in the activation process
rather than ligand binding. This is further supported by the
observation of a reduction in efficacy with little or no effect on
the affinity of the ligands and the decreased efficacy in the
mutated H1 receptor (Leurs et al., 1995).

We also investigated the mutations using the FLIPR, in
which the H3 and mutant receptors are coupled to the phos-
phoinositide pathway through a chimera of Gαq, containing
the five C-terminal amino acids of Gai (Conklin et al., 1996).
In general, the FLIPR data are in agreement with the func-
tional adenyl cyclase results. However, the agonist potencies
determined in the FLIPR are lower than in the cAMP assays,
implying that there is a less efficient interaction between
receptor and the chimeric G protein compared with the na-
tive G protein α-subunit, Gai. This also suggests that motifs
other than the five C-terminal amino acids are also impor-
tant for this interaction. Accordingly, roles in receptor-G
protein coupling have been suggested for the N termini of
both Gαq and Goα (Kostenis et al., 1997; Ho and Wong,
2000). In the FLIPR, the effects of the mutations are much
more dramatic. For instance, in this assay the E206A muta-
tion results in a 65-fold increase in the half-maximally effec-
tive concentration of histamine compared with the 22-fold
difference seen in the cyclase assay. The less efficient cou-
pling of the receptor to the chimeric Gα subunit may be
responsible for this, as well as the observed reduction of the
efficacy of some ligands. In this assay, histamine was a full
agonist, but the selective H3 agonist imetit appears to have
partial agonist activity. Interestingly, in the FLIPR, impen-
tamine was unable to stimulate the wild-type H3 receptor,
ethough it is a potent agonist at the wild-type receptor in
the cAMP assay. However, impentamine was able to stimu-
late the E206A receptor but with a lower efficacy than the
other ligands. These observations demonstrate that the ago-
nist-receptor complexes formed by the three different ago-
nists are not equivalent in their ability to activate the G
protein and suggest that different agonists may promote
different conformational states of the receptor.

The multiplicity of agonist-receptor complexes formed by
different agonists may be responsible for signal trafficking
(Kenakin, 1995), the term given to the ability of agonists to
differentially stimulate the coupling of a receptor to different
signaling pathways as described for the serotonin 2A and 2C
receptors (Berg et al., 1998). The promiscuous coupling of
the H3 receptors to the Gaq/Gai chimera in the FLIPR provides
a platform with which to identify the different agonist-recep-
tor complexes, allowing us to study the more sensitive and
subtle effects of receptor site-directed mutagenesis and li-
gand structure-activity relationships. The inability of impen-
tamine to stimulate the wild-type receptor in the FLIPR
assay, despite its full agonist activity at the wild-type recep-
tor in the cAMP assay, suggests that its agonist-receptor
complex is fundamentally different from that of other full
agonists, such as histamine. It is possible that on interaction
with the wild-type receptor, impentamine may form an ago-
nist-receptor complex of lower intrinsic potency. A weaker
agonist-receptor complex could also explain why impentam-
nine is unable to activate the H3 receptor pathways in the
guinea pig ileum (Leurs et al., 1996), although in this case a
different receptor subtype cannot be ruled out.

In conclusion, we have identified TM5 residues that affect
the function of the H3 receptor. Although there is little pri-
mary sequence homology, these residues align with those
that have previously been shown to be important for the
function of the histamine H1 and H2 receptors and other
members of the biogenic amine receptor family. Moreover,
our data suggest that different H3 receptor agonists may
promote different receptor conformations.

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Ho MKC and Wong YH (2000) The amino terminus of galpha z is required for
receptor recognition, whereas its alpha 4/beta 6 loop is essential for inhibition of


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