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Preliminary communication

Modeling of the agonist binding site of serotonin human 5-HT_{1A}, 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors

DH Bremner, NS Ringan, G Wishart

Division of Chemistry, School of Molecular and Life Sciences, University of Abertay Dundee, Bell Street, Dundee, DD1 1HG, UK

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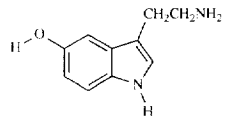
Summary — Three dimensional models for the serotonin human 5-HT_{1A}, 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors have been constructed utilising the coordinates of bacteriorhodopsin as an initial template. The 5-HT receptor agonists 5-hydroxytryptamine, 8-hydroxy-2-(di-*n*-propylamino)tetralin, 5-carboxamidotryptamine, 5-hydroxy-*N,N,N*-trimethyltryptamine and sumatriptan have been docked into the probable agonist binding site of the receptor models between transmembrane domains 3, 4, 5 and 6 in order to validate the proposed models. Detailed interactions of the ligand–receptor complexes are reported. The relative binding affinities of the ligands can be accounted for, in a qualitative sense, from an interpretation of the ligand–receptor interactions.

5-HT receptor / serotonin / modeling / agonist

Introduction

Serotonin (5-HT) **1** is a neurotransmitter which is believed to participate in a number of pathological conditions and disorders [1]. In recent years there has been an upsurge of interest in the 5-HT receptors due to the identification and cloning of new 5-HT receptors [2] and an interest of 5-HT in migraine headache [3, 4]. The discovery of the antimigraine drug Sumatriptan **2**, which was found to bind with high affinity to the 5-HT_{1D} sub-population of receptors [5–8] has made the 5-HT_{1D} receptors important targets in the development of novel selective antimigraine drugs [9–11].



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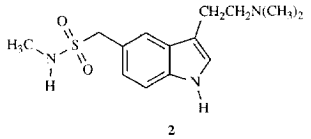

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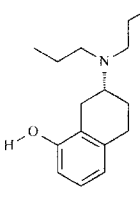

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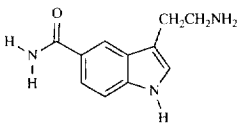

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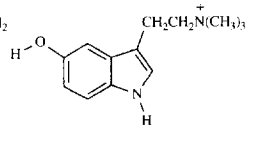

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This study attempts to produce quickly, working molecular models for the 5-HT_{1D} receptor agonist binding sites. This will allow the identification of the amino-acid residues which may take part in the binding of agonists. This is concerned with a current study of novel heterocycles within the proposed agonist binding site of these receptors with a view to future synthesis. The 5-HT receptors (with the exception of the 5-HT_3 receptor which is a ligand-gated ion channel) belong to the superfamily of G-protein-coupled receptors (GPCRs). These are membrane-embedded receptors that interact with their respective secondary messengers via a guanine-nucleotide binding protein. Although there are, as yet, no three-dimensional crystal structure data available for GPCRs, the generally accepted view is that GPCRs contain seven antiparallel α-helical transmembrane domains with an extracellular *N*-terminus and cytoplasmic C-terminus [12, 13]. There have been many molecular models reported in recent literature [14, 15] most using the three-dimensional electron cryo-microscopy structure of bacteriorhodopsin [16]. Although bacteriorhodopsin is not G-protein coupled and shows very little sequence homology to the GPCRs, it does consist of seven antiparallel transmembrane helices arranged anticlockwise as viewed from the extracellular surface. Visual rhodopsin, which is a GPCR, and bacteriorhodopsin have a number of structural features in common and thus may share the same fold [17]. Alternatively some models [18] have used the

two-dimensional projection density map of the GPCR bovine rhodopsin [19] and more recently a low resolution structure of bovine rhodopsin determined by electron cryo-microscopy has been published [20]. It was also shown that although bovine rhodopsin does have the same overall topology as that of bacteriorhodopsin the positioning and tilt of some of the transmembrane helices may well be different. Although there have been many modeling studies of GPCRs, very few actually examine the agonist binding site reporting the interactions of agonists other than that of the natural ligand [15, 21]. The ligand binding site for the cationic monoamine receptors is believed from mutagenesis data [22–24] to involve a conserved aspartate residue on helix 3. Almost all models of the monoamine ligand–receptor interactions show the agonist binding site to be between helices 3, 4, 5 and 6 allowing the cationic amino group of the ligand to interact with the conserved aspartate residue upon transmembrane helix 3. Furthermore an aspartate residue upon helix 2 has likewise been shown from mutagenesis to be important but it is generally thought that it may be necessary in allosteric activation of the G-protein [23].

Approach adopted

We have constructed models of the 5-HT_{1A}, 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors using the template of bacteriorhodopsin. Although there are known limitations to this approach it does appear to be highly likely that the structures of GPCRs and bacteriorhodopsin are similar enough to justify the use of bacteriorhodopsin coordinates at least as an initial template for the arrangement of the transmembrane domains until full coordinates of bovine rhodopsin become available in the public domain. The 5-HT_{1A} receptor was constructed in the first instance since there is more binding affinity and mutagenesis data available for this receptor relative to the other 5-HT_1 subtype receptors. This model was used as a prototype upon which models of the 5-HT_{1D} receptors could be constructed.

Methods

Sequence alignment

The amino-acid sequences of the human $5HT_{1A,1D\alpha,1D\beta,1E,1F}$ and the 5-HT_{2A} and 5-HT_{2C} receptors [7, 25–28] were extracted from the Swissprot sequence database and read into the InsightII [29] molecular modeling package. The sequences were aligned using the Dayhoff PAM 120 evolutionary scoring matrix [30]. The proposed α-helical transmembrane domains are known to be hydrophobic in nature thus a hydrophobicity analysis should give the approximate regions of the seven helices. This analysis was performed using the scale of Kyte and Doolittle [31] over a window size of 5 and hydrophobicity threshold of 0. These values were found to be optimal from analysis of the bacteriorhodopsin sequence data and cross-referencing this with the three-dimensional structure. When applied to the 5-HT receptors under consideration this resulted in six distinct hydrophobic regions corresponding to helices 1–6 for each of the seven receptors studied. Helix 7 was less well defined, therefore data from the sequence homology had to be taken into account. The transmembrane domains are regions of high sequence conservation thus the hydrophobicity data coupled with that of the sequence homology allowed the determination of the positions of the seven α-helices (fig 1). It is however stressed that these will only be approximate positions of the helices and it should be noted that there are sequence gaps in two of the helices. Insertions and deletions are unlikely in structurally conserved regions and it could be said that this alignment is wrong. However, the gaps occur towards the C-terminus of each helix and may be caused by errors in the sequence alignment or it may be the case that there are slight errors in the length or location of the transmembrane helices. Nevertheless these discrepancies will have very little bearing upon the agonist binding site.

Helical wheel projections

The α-helices of each receptor were plotted around helical wheels at intervals of 100° using the GCG program Helicalwheel [32]. The helical wheels were arranged upon the template of bacteriorhodopsin and the rotational position of each helix was evaluated according to three factors: (a) hydrophilic and charged residues were directed towards the central cleft; (b) conserved residues were directed towards the central cleft; and (c) residues proposed to be involved in ligand binding from mutagenesis data were directed towards the central cleft.

Construction of the helices

The α-helices of the 5-HT_{1A} and 5-HT_{1D} receptors were constructed from the Biopolymer module within Insight with dihedral angles of $\Phi = -65°$, $\Psi = -40°$ and $\omega = 180°$. A number of the helices contain proline residues which tend to induce kinking of the helical backbone. It is thought that this kinking is an important structural feature of the receptor therefore kinks were introduced into the required helices according to the work of Polinsky and coworkers [33] who studied minimum energy conformations of Ala_8-Leu-Pro-Phe-

	HELIX 1	HELIX 2
5-HT$_{1A}$	VITSLLLGTLIFCAVLGNACVVAAIA	YLIGSLAVTDLMVSVLVLPMAAL-YQV
5-HT$_{1D\alpha}$	ISLAVVLSVITLATVLSNAFVLTTIL	YLIGSLATTDLLVSILVMPISIA-YTI
5-HT$_{1D\beta}$	VLLVMLLALITLATTLSNAFVIATVY	YLIASLAVTDLLVSILVMPISTM-YTV
5-HT$_{1E}$	MLICMTLVVITTLTTLLNLAVIMAIG	YLICSLAVTDLLVAVLVMPLSII-YIV
5-HT$_{1F}$	ILVSLTLSGLALMTTTINSLVIAAII	YLICSLAVTDFLVAVLVMPFSIV-YIV
5-HT$_{2A}$	NWSALLTAVVIILTIAGNILVIMAVS	YFLMSLAIADMLLGFLVMPVSMLTILY
5-HT$_{2C}$	NWPALSIVIIIIMTIGGNILVIMAVS	YFLMSLAIADMLVGLLVMPLSLLAILY

HELIX 3	HELIX 4
VTCDLFIALDVLCCTSSILHLCAIAL	AAALISLTWLIGFLISIP-PML
ILCDIWLSSDITCCTASILHLCVIAL	AATMIAIVWAISICISIP-PLF
VVCDFWLSSDITCCTASLIHLCVIAL	AAVMIALVWVFSISISLP-PFF
FLCEVWLSVDMTCCTCSILHLCVIAL	AALMILTVWTISIFISMP-PLF
VVCDIWLSVDITCCTCSILHLSAIAL	AGIMITIVWIISVFISMP-PLF
KLCAVWIYLDVLFSTASIMHLCAISL	AFLKIIAVWTISVGISMPIPVF
YLCPVWISLDVLFSTASIMHLCAISL	AIMKIAIVWAISIGVSVPIPVI

HELIX 5	HELIX 6
YTIYSTFGAFYIPLLLMLVLY	KTLGIIMGTFILCWLPFFIVALVLPF
YTIYSTCGAFYIPSVLLIILY	KILGIILGAFIICWLPFFVVSLVLPI
YTVYSTVGAFYFPTLLLIALY	KTLGIILGAFIVCWLPFFIISLVMPI
YTIYSTLGAFYIPLTLILILY	RILGLILGAFILSWLPFFIKELIVGL
STIYSTFGAFYIPLALILILY	TTLGLILGAFVICWLPFFVKELVVNV
FVLIGSFVSFFIPLTIMVITY	KVLGIVFFLFVVMWCPFFITNIMAVI
FVLIGSFVAFFIPLTIMVITY	KVLGIVFFVFLIMWCPFFITNILSVL

HELIX 7
LGAIINWLGYSNSLLNPVIYAY
LFDFFTWLGYLNSLINPIIYTV
IFDFFTWLGYLNSLINPIIYTM
VADFLTWLGYVNSLINPLLYTS
MSNFLAWLGYLNSLINPLIYTI
LLNVFVWIGYLSSAVNPLVYTL
LLNVFVWIGYVCSGINPLVYTL

Fig 1. Sequence alignment of the seven transmembrane domains of the human 5-HT receptors.

Ala_8 at a dielectric constant of 4. The C-terminus of each helix was capped with an *N*-methyl group and the *N*-terminus with an acetyl group. The potential types and partial charges were fixed using the set of rules accompanying the CVFF forcefield [34] as supplied with Discover [35]. Each helix was subjected to 200 steps of steepest descent energy minimisation, without using cross terms, followed by 1000 steps of

conjugate gradient minimisation using cross terms. All minimisations and subsequent minimisations were carried out at a dielectric constant of 4 using the CVFF forcefield parameters. The mean RMS deviation of the helical backbone after minimisation was 0.998 Å. The loop regions and the two terminal regions were not constructed since the conformations of these regions are not known and ligand binding is believed to occur in the transmembrane domain [36].

Construction of the 5-HT$_{1A}$ receptor

The seven helices of the 5-HT$_{1A}$ receptor were superimposed onto the template of bacteriorhodopsin. This resulted in a crude initial model upon which refinement procedures could be carried out. The helices were rotated and translated about their helical axis to obtain a model consistent with that of the helical wheel data and to maximise interhelical interactions. Sidechain conformations of the residues were checked with reference to the standard rotamer library as supplied with Insight. The receptor was initially energy minimised for 1000 iterations of steepest descent followed by 2000 iterations of conjugate gradient minimisation; all backbone atoms were fixed during the minimisation. This was followed by a molecular dynamics simulation of 10 ps initialisation and 100 ps simulation at 310 K with a timestep of 1 fs, again with backbone atoms fixed. Non-bonded cutoffs were used at 12 Å for the dynamics and minimisation stages. The simulation period was divided into 10 ps intervals and the average conformations over these intervals were calculated. Finally the conformations were energy minimised for 1000 steps of steepest descent followed by 2000 steps of conjugate gradient again using 12 Å cutoffs and similarly the backbone atoms were fixed. The minimum energy conformation was retained for further analysis.

Construction of the 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors

There were three possible methods by which these receptors could be modeled. In *Method 1*, the receptors could have been superimposed onto the backbone of bacteriorhodopsin and the receptor built in the same manner as the 5-HT$_{1A}$ receptor. In *Method 2*, the receptors could have been built from the exact backbone of the previously constructed 5-HT$_{1A}$ receptor by mutating the required residues. Finally, in *Method 3* the 5-HT$_{1A}$ receptor could be used as the template upon which the helices of the 5-HT$_{1D}$ receptors could be superimposed. *Method 1* would possibly have resulted in models that were quite distant in terms of backbone atom deviation from the 5-HT$_{1A}$ receptor. *Method 2* would give the impression of forcing the backbone of the 5-HT$_{1D}$ receptors to adopt the exact conformation of the 5-HT$_{1A}$ receptor, therefore we chose *Method 3*. The transmembrane helices of the 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors were superimposed using their backbone atoms onto the prototype 5-HT$_{1A}$ receptor. Sidechain conformations were checked prior to structure refinement and the models were subjected to the same minimisation and dynamics procedure as for the 5-HT$_{1A}$ receptor. The RMS gradient of the minimised receptors was less than 0.001 kcal/Å. The RMS deviances in backbone structures were subsequently calculated (table I). These results are consistent with the sequence alignment, in that 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ show greater similarity with each other rather than with the 5-HT$_{1A}$ receptor.

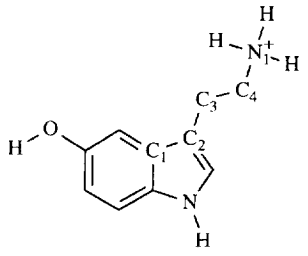
Construction of receptor ligand complexes

5-Hydroxytryptamine

5-HT **1** was assembled within Insight, partial charges were assigned and the molecule was energy minimised to a gradient of less than 0.001 kcal/Å to obtain the two global minimum energy conformations previously calculated (Wishart and Ringan, unpublished results). These were named 5-HT(A) and 5-HT(B) (fig 2). The ligands were subsequently docked into the 5-HT receptors in a variety of positions allowing the protonated amine group of the ligand to interact with the proposed important aspartate residue found on helix 3 protruding into the central cleft of the receptor. The hydroxyl group of 5-HT was positioned in such a way that it could potentially interact with a number of serine or threonine residues upon helices 4, 5 and 6. The conformations of the receptor sidechains were checked with the standard rotamer library to ensure there were no sidechains wrongly positioned in high energy areas. The ligand receptor complexes were then energy minimised for 1000 iterations of steepest descent followed by 2000 iterations of conjugate gradient. Cutoffs were applied at 12 Å and the backbone atoms were fixed in position.

Table I. RMS deviations between receptor model backbone atoms.

Receptor	*Backbone (RMS)*
5-HT$_{1A}$/5-HT$_{1D\alpha}$	0.77 Å
5-HT$_{1A}$/5-HT$_{1D\beta}$	0.76 Å
5-HT$_{1D\alpha}$/5-HT$_{1D\beta}$	0.42 Å



CONFORMATION	D1 (C_1-C_2-C_3-C_4)	D2 (C_2-C_3-C_4-N_1)
5-HT(A)	80.33°	-178.40°
5-HT(B)	-80.33°	178.40°

Fig 2. Global minimum energy conformations of protonated 5-HT in terms of dihedrals D1 (C_1-C_2-C_3-C_4) and D_2 (C_2-C_3-C_4-N_1).

8-Hydroxy-2-(di-n-propylamino)tetralin

8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) is a selective 5-HT_{1A} agonist [37, 38] (table II). Although the two enantiomers of 8-OH-DPAT both have similar binding affinities at the 5-HT_{1A} receptor site it has been shown that only the *R*-enantiomer is a full agonist whereas the *S*-enantiomer shows only partial agonist characteristics [39]. Therefore, this study will concentrate solely upon the *R*-enantiomer **3**. The tetralin ring structure was built and geometry-optimised within Insight yielding two distinct ring conformations. These conformations were found to be consistent with conformations found for the tetralin ring moiety within the CSSR database [40, 41]. The required substituents were added and the molecule was protonated. Conformational analysis was performed at a 30° increment of the two *n*-propyl substituents. Each conformer was geometry optimised to an RMS gradient of less than 0.001 kcal/Å. The lowest energy conformations for each of the ring structures were named 8-OH-DPAT(A) and 8-OH-DPAT(B), with the latter being the global minimum energy conformation by 3 kcal/mol. Both conformations of 8-OH-DPAT were docked into the 5-HT receptor models by superposition of the phenol moiety of 8-OH-DPAT onto that of 5-HT in the final, energy-minimised 5-HT-receptor complex. The 8-OH-DPAT-receptor complexes were minimised in exactly the same manner as for the 5-HT-receptor complexes with the exception that cutoffs of 18 Å were used due to the increase in size of the ligand.

Table II. Binding affinities of agonists at 5-HT receptors.

Ligand	*Binding affinity (pK_i)*		
	5-HT_{1A}[a]	*5-$HT_{1D\alpha}$*[b]	*5-$HT_{1D\beta}$*[b]
5-HT	8.38	8.41	8.37
8-OH-DPAT	8.61	6.92	6.59
5-CT	9.53	9.15	8.80
5-OH-TMT	na	na	na
Sumatriptan	6.62	8.47	8.11

[a]5-HT_{1A} binding data from reference [42]; [b]5-HT_{1D} binding data from reference [8]; na: no binding figures available but 5-OH-TMT is known to bind at 5-HT_{1A} receptors with an affinity several-hundred-fold lower than 5-HT [43].

5-Carboxamidotryptamine and 5-hydroxy-N,N,N-trimethyltryptamine

5-Carboxamidotryptamine (5-CT) **4** is a high affinity 5-HT receptor agonist, but it is non-selective over the 5-HT_1 subtype of receptors (table II). 5-Hydroxy-*N,N,N*-trimethyltryptamine (5-OH-TMT) **5** binds at 5-HT_{1A} sites with an affinity several-hundred-fold lower than that of 5-HT itself. Both molecules were constructed and 5-CT was protonated. Both ligands were geometry optimised as for 5-HT to give each molecule two minimum energy conformations which have the alkyl amino substituent in a similar position to that of the optimised conformations of 5-HT. The two conformations of 5-CT were docked into all the 5-HT receptor models while 5-OH-TMT was docked only into the 5-HT_{1A} receptor. All conformations were docked into a position similar to that of the final, energy-minimised 5-HT-receptor complex. The ligand–receptor models were all energy minimised using exactly the same criteria as for the 8-OH-DPAT–receptor complexes.

Sumatriptan

Sumatriptan **2** was constructed, protonated and geometry optimised to give two low energy conformations A and B similar to those described for 5-HT. The C-5 position of sumatriptan however contains three rotatable bonds which will dictate the position of the substituent. A conformational analysis was performed about these bonds using a 30° increment. Subsequent geometry optimisation to an RMS gradient of less than 0.001 kcal/Å yielded two sets of 13 minimum energy structures differing in energy by 6.8 kcal. The 26 conformers were docked into the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors in a position equivalent to that of 5-HT at the respective receptors. Only the most

promising conformations of the ligand from the sumatriptan–5-HT_{1D} receptor complexes were docked into the 5-HT_{1A} receptor. All ligand–receptor complexes were energy minimised as described previously for 8-OH-DPAT.

Results and discussion

(Residues will be referred to by their three letter code, followed by the respective position of the residue in the helix followed finally by the helix number in parentheses.)

The 5-HT–receptor complex

The results from the extensive docking experiments using 5-HT were analysed in terms of ligand–receptor interactions and relevance towards the experimental data. Interaction energies were assumed to be less important and hence the final results obtained are not the most energetically favourable orientations of the ligand within the active site but they did prove to be the most logical in terms of the experimental data available. The observed 5-HT binding site of the 5-HT_{1A} (fig 3), 5-$HT_{1D\alpha}$ (fig 4) and 5-$HT_{1D\beta}$ (fig 5) receptors is in a pocket defined by helices 3, 4, 5 and 6. It is also possible that helix 7 may participate in the binding of some ligands. The ligand is positioned approximately 15 Å into the cleft from the extracellular opening. The aromatic nucleus of 5-HT is anchored by two conserved aromatic residues Trp9(H4) and Phe18(H6) which create favourable perpendicular interactions with the indole moiety of the ligand. The cationic amine group forms an ion pair with Asp10(H3) (mean distance NH···OC 2.01 Å), this interaction being further stabilised by a pocket of hydrophobic aromatic residues surrounding the ion pair. These residues consist of Phe/Trp6(H3), Phe17(H6) and Trp14(H6) which forms the base of the hydrophobic pocket and can form additional interactions with the aromatic indole ring. Hydrogen bonds are formed between the 5-hydroxyl group of the ligand which accepts a hydrogen from Thr6(H5) (mean distance OH···O 1.93 Å), this residue is mutated for a serine in the 5-HT_2 subtype of receptors. The nitrogen of the indole nucleus forms a hydrogen bond with Ser12(H4) in the case of the 5-HT_{1D} receptors but in the 5-HT_{1A} receptor this residue is mutated for a glycine. It is also possible that the oxygen of the hydroxyl group of the 5-HT_{1D} receptors interacts with Ser21(H6) although the mean hydrogen bond angle of 114° is not ideal. One of the most interesting results to arise from these docking studies is that both conformations of 5-HT appear to be accepted into the active site with minimum overlap of ligand and receptor. A closer analysis taking into account nonbonded interaction energies between ligand and receptor showed that, of the two conformations of 5-HT, the 5-HT_{1A} receptor showed a distinct preference for conformation A. This is in contrast with the 5-HT_{1D} receptors which show greater interaction energies for conformation B.

The 8-OH-DPAT–receptor complex

Within the 5-HT_{1A} receptor (fig 6) both conformations of the ligand show the hydrogen bond donated from Thr6(H5) to the hydroxyl substituent at the C8 position. The ligand is accommodated in the binding site that was identified for 5-HT with only minor adjustments in the sidechains of the aromatic residues

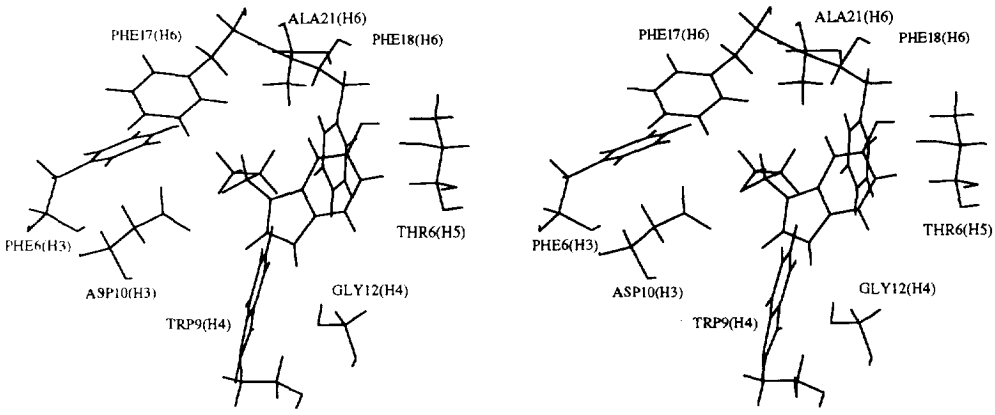


Fig 3. 5-HT docked within the agonist binding site of the 5-HT_{1A} receptor model showing residues involved in binding.

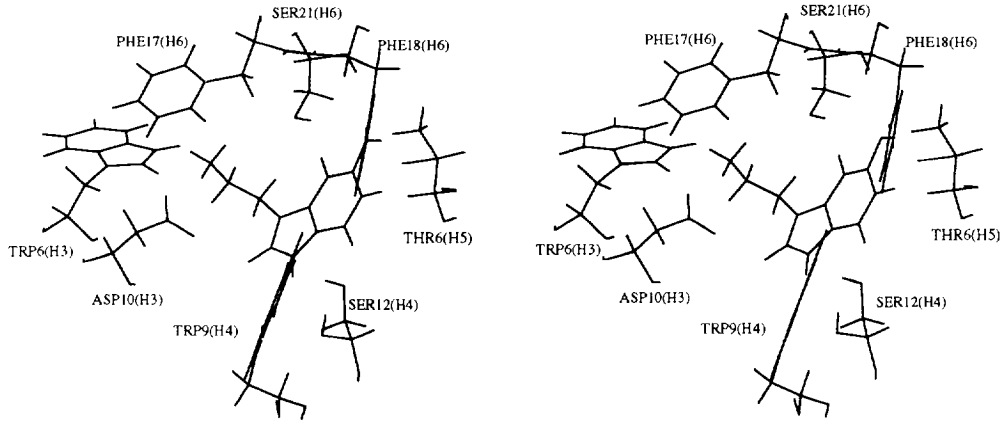


Fig 4. 5-HT docked within the agonist binding site of the 5-$HT_{1D\alpha}$ receptor.

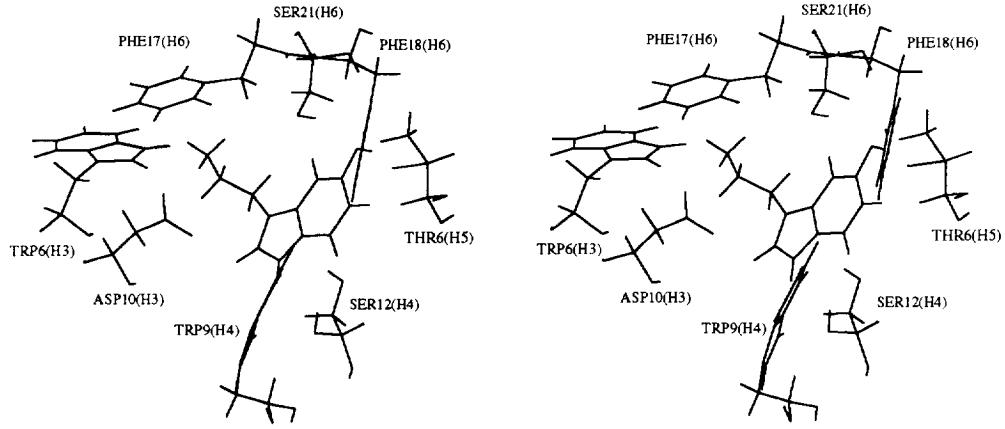


Fig 5. 5-HT docked within the agonist binding site of the 5-$HT_{1D\beta}$ receptor.

surrounding the bulky *n*-propyl groups of 8-OH-DPAT. It appears that the aromatic residues surrounding the sidechain, Trp14(H6), Phe17(H6) and Phe6(H3) can adjust their conformation very slightly to allow the process of ligand binding. The results from the 5-HT_{1D} receptors show quite different pictures. The ligand in all cases has moved down into the central cleft by approximately 1–2 Å due to the steric repulsion of the larger tryptophan residue Trp6(H3) with one of the *n*-propyl groups. This residue is mutated to phenylalanine in the 5-HT_{1A} receptor which, with a slight adjustment of the residue sidechain, can accommodate the *n*-propyl substituents comfortably. The movement of the ligand within the 5-HT_{1D} binding sites has altered the hydrogen bond of the hydroxyl group as it now donates the hydrogen to Thr6(H5). There is also a greater degree of steric bumping between the tetralin ring structure with Ser12(H4). This residue is conserved in all the 5-HT receptors with the exception of the 5-HT_{1A} receptor where a glycine is found. This suggests that Ser/Gly12(H4) and Trp/Phe6(H3) play an important role in the fit and selectivity of 8-OH-DPAT at the 5-HT receptors. The steric bumping observed between 8-OH-DPAT and the 5-HT_{1D} receptors has caused the movement of the ligand in the binding site and this may account for the lesser affinity of the ligand at these receptors.

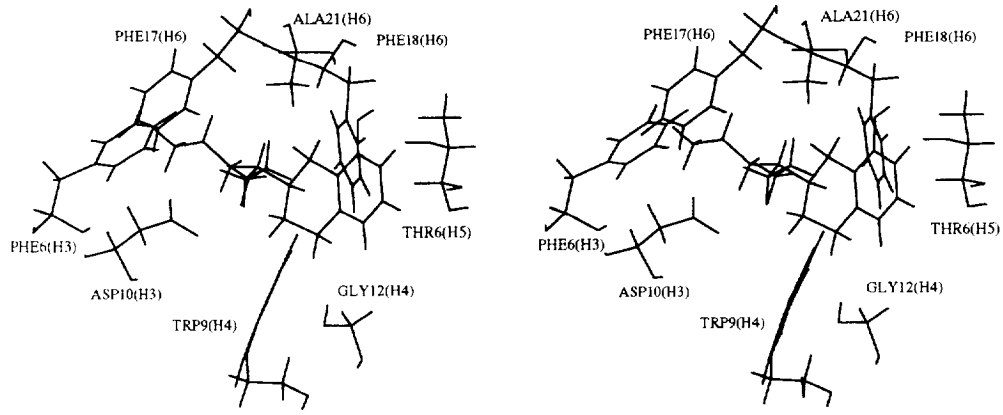


Fig 6. Stereoview of 8-OH-DPAT binding to the 5-HT_{1A} receptor.

The 5-CT–receptor complex

Docking studies of 5-carboxamidotryptamine at the 5-HT_{1A} receptor showed that both conformations remained in the same position as 5-HT; similar results were obtained for the 5-$HT_{1D\alpha/1D\beta}$ receptors (fig 7). In all receptors the carbonyl group of the ligand was well positioned to accept a hydrogen bond from Thr6(H5). 5-CT within the 5-HT_{1D} receptors can additionally interact with Ser21(H6). As with 5-HT, the aromatic nucleus was held in position by Trp9(H4) and Phe18(H6). The nitrogen of the indole ring formed a hydrogen bond with Ser12(H4) of the 5-HT_{1D} receptors. Again similar to the results for the 5-HT–receptor complexes the 5-HT_{1A} receptor showed greater interaction energy with the conformation similar to that of 5-HT(A). The 5-HT_{1D} receptors showed preference for the mirror image conformation B.

The 5-OH-TMT–receptor complex

Initial analysis of these complexes shows a similar position of the ligand to that observed for 5-HT, with formation of the hydroxyl-Thr6(H5) hydrogen bond. However a closer inspection yields some interesting findings. The two conformations show slightly different results since in one case a methyl group has caused a conformational change in the highly important Asp10(H3) residue. The other complex shows no change in the aspartate residue but the conformation of the sidechain of the ligand has become extended. In both cases electrostatic interaction energies between the ligand and Asp10(H3) were reduced by approximately 20 kcal/mol. In conclusion, the results observed highlight the tightness of fit between the ligand and the receptor. This has induced conformational changes in either the receptor or

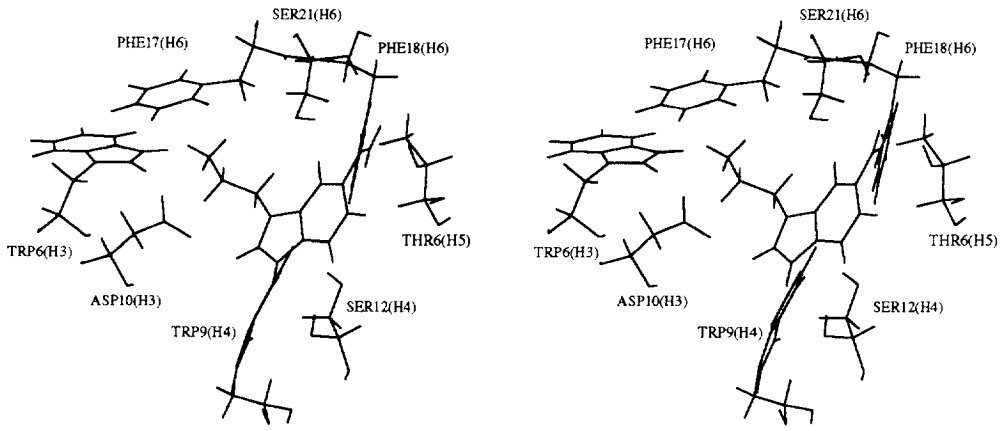


Fig 7. Stereoview of 5-CT binding to the 5-$HT_{1D\beta}$ receptor.

ligand leading to a reduction in the electrostatic energy which may explain the reduced binding affinity of the ligand.

The sumatriptan–receptor complex

All but one of the sumatriptan conformations produced major conformational changes in both ligand and receptor in the 5-HT_{1D} binding pocket. Sumatriptan was accommodated when the C-5 substituent was folded back across the indole moiety (fig 8). This allows the nitrogen of the substituent to accept a hydrogen bond from Ser21(H6) and one of the oxygens of the sulphone group to accept a hydrogen bond from Thr6(H5). The larger substituent has resulted in a slight movement of the indole nucleus down the central cleft. The nitrogen of the aromatic group can still interact with Ser12(H4) and due to the slight repositioning of the ligand, can form additional interactions with the conserved Ser16(H4). The indole ring is again anchored by the aromatic residues Phe18(H6), Trp9(H4) and Trp14(H6). There is however a minor reorganisation of the aromatic residues surrounding the protonated nitrogen of sumatriptan and Asp10(H3). The sidechains of Phe17(H6), Phe2(H7) and Phe5(H7) have all undergone a slight change in conformation to accommodate the two methyl groups of the ligand. The sumatriptan–5-HT_{1A} receptor complex shows contrasting results. The ion pair between Asp10(H3) and the protonated group of the ligand is again formed but the mutation of Ser21(H6) to alanine does not allow the C-5 substituent to be held in position and thus stabilise the conformation of sumatriptan. The non-polar Ala21 (H6) does not interact favourably with the polar sidechain of the ligand resulting in the ligand repositioning itself within the binding site weakening the sulphone–Thr6(H5) hydrogen bond and the interaction of the ligand with Asp10(H3). The combination of these factors may account for the decreased affinity of sumatriptan for the 5-HT_{1A} receptor.

Evaluation of the binding site

The results of this study appear to be consistent with available binding and mutagenesis data. It has been shown how the agonists studied all interact with Asp10(H3) which is known from mutagenesis studies to affect the agonist binding affinity of the 5-HT_{1A} and 5-HT_{2A} receptors. Mutations of Thr6(H5) and Phe18 (H6) also diminish agonist affinities for the 5-HT_{1A} and 5-HT_{2A} receptors respectively [22, 23] both residues having an important role in the three receptor models. The models account for the binding of 5-HT and 5-CT. The lack of affinity towards the 5-HT_{1A} receptor shown by 5-OH-TMT is explained by a decrease in the electrostatic interaction energy caused by steric bumping and conformational change in the receptor. The selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, does not contain the indole ring system and the 5-HT_{1A} receptor has no residue with the potential to interact with the pyrrole moiety of the indole ring, ie, Gly12(H4). The bulky sidechain is also tolerated possibly due to Phe6(H3) which is slightly smaller than the tryptophan residue observed for the remaining 5-HT receptors. The binding of sumatriptan has some interesting implications. It seems likely that Ser21(H6) of the 5-HT_{1D} receptors can, along with Thr6(H5) hold the amino-sulphone group into position. Mutation of Ser21(H6) to Ala as observed for the 5-HT_{1A} receptor may create repulsion of the C5 substituent of the ligand and ultimately lead

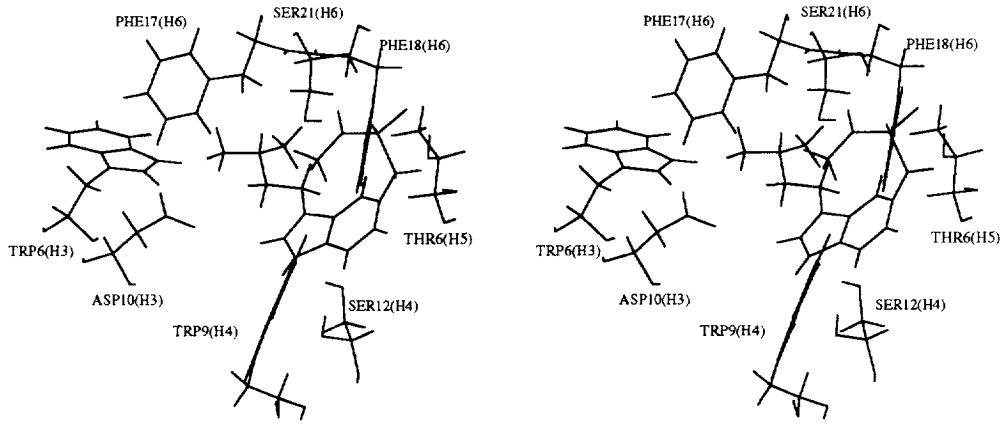


Fig 8. Stereoview of sumatriptan binding to the 5-$HT_{1D\alpha}$ receptor.

to a reduced binding affinity. It has also been shown by a number of independent research groups that the mutation of Thr6(H7) to Asn (Asn is found in the 5-HT_{1A} and rodent 5-HT_{1B} receptors) decreases the affinity of sumatriptan [44–46]. This would possibly imply a direct role of this residue in the binding of the ligand. Analysing the present model the threonine residue was found to be a distance of approximately 8–10 Å away from the ligand. The threonine residue however can form a hydrogen bond to the backbone of Phe2(H7) whereas the asparagine of the 5-HT_{1A} receptor cannot. It is particularly interesting that this threonine residue is directly above a glycine residue Gly9(H7) which is conserved in the serotonergic, adrenergic, dopaminergic and histamine receptors. The glycine residue may act as a hinge allowing the helix to change conformation but unfortunately this cannot be shown in the present model since backbone atoms were fixed during minimisations. Therefore it seems plausible that the conformational changes of Phe17(H6), Phe5(H7) and Phe2(H7) which take place upon the binding of sumatriptan will affect the conformation of helix 7. The movement of this helix can be stabilised by a hydrogen bond between Thr6(H7) and the backbone of Phe2(H7) in the 5-HT_{1D} receptors. There is no equivalent hydrogen bond observed for the 5-HT_{1A} receptor and this can explain the importance of Thr/Asn6(H7) upon the binding affinity of sumatriptan. This proposal of the function of Thr6 (H7) for the 5-HT_{1D} receptors is supported by Smolyar and Osman [21] who studied the interactions between sumatriptan and the 5-$HT_{1D\alpha}$ receptor, although the proposed binding site of the ligand differs from that of the present study. It may be suggested in the rhodopsin-derived GPCR models [18] where helix 7 is closer to helix 3 and the probable agonist binding site would allow direct interaction of Asn/Thr6(H7) with the agonist. This suggestion was tested by translation of transmembrane helix 7 of the sumatriptan bound 5-HT_{1D} receptors into the central cleft closer to helices 2 and 3. The distance between Thr6(H7) and sumatriptan had decreased but was still in the region of 4–5 Å. This appears to support the hypothesis that Thr6(H7) plays an indirect role in the binding and selectivity of sumatriptan at the 5-HT_{1D} receptors.

The fact that both global minimum energy conformations of 5-HT and similar conformations of 5-CT and sumatriptan can occupy the same agonist binding site suggests two possible explanations. The ligand binding process may allow the conformation of the ligand sidechain to alter during the binding and activation of the receptor or alternatively different conformations of the ligands may bind to different sub-populations of the 5-HT receptors. The presented binding site models are undoubtedly similar to that of Hibert et al [14, 47] incorporating the aspartate on helix 3 and threonine upon helix 5. However in the models of Hibert the aromatic portion of the agonists are anchored by Phe10(H5) whereas the presented model suggests that this residue is directed not into the central binding cleft but towards helix 6 and indeed can stack against the more important Phe18 (H6) which does interact directly with the indole moiety of the agonists. Hibert also makes no mention of Ser21(H6) and Thr6(H7) both of which are apparently important in the binding and selectivity of sumatriptan at the 5-HT_{1D} receptors.

Conclusions

The study to date has proposed models for the 5-HT_{1A}, 5-$HT_{1D\alpha}$ and the 5-$HT_{1D\beta}$ receptors. The binding of a number of agonists has been studied, not so much as in terms of energies but in a more qualitative sense looking at the position of the bound ligands. These models are consistent with experimental mutagenesis and ligand binding data. The models account for the high affinities of 5-HT and 5-CT, the selectivity of 8-OH-DPAT and sumatriptan and the low affinity of 5-OH-TMT at the 5-HT_{1A} receptor. The models of this study appear to show encouraging results upon consideration of the experimental data available, however one should remember that they are only models. Molecular modeling of GPCRs is still a speculative procedure and there are almost certainly inaccuracies in all models constructed. We have provided a geometrical picture of the agonist binding site pocket of the 5-HT receptors considered. The amino-acid residues within the binding site region have been identified and this will prove a valuable guide in our attempt to design novel heterocyclic analogues of 5-HT as potential 5-HT receptor agonists.

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