Binding-Site Modeling of the Muscarinic m1 Receptor: A Combination of Homology-Based and Indirect Approaches

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A model of the muscarinic m1 receptor has been constructed on the basis of the putative three-dimensional structural similarity between bacteriorhodopsin and G-protein coupled receptors. The homology-based m1 receptor model takes into account hydrophobicity and conserved amino acids and information from site-directed mutagenesis studies and from hydropathy plots. The resulting model was used in conjunction with an indirect model which describes a proposed active agonist conformation of acetylcholine and a number of related compounds. A receptor-excluded volume was constructed by superimposing these muscarinic agonists and calculating their combined van der Waals volume. The resulting m1 receptor excluded volume was used to define the agonist binding site, which consists of nine amino acids and which binds agonists primarily through interaction with Asp105 (ionic interaction), Thr192 and Asn382 (hydrogen bonds). The model is flexible since the conformation of the nine amino acids may change in response to the agonist structure. The combination of indirect and homology-based approaches is particularly attractive since it utilizes more experimental data than a purely homology-based model and since a binding-site model might be more realistic and general in terms of applicability than indirect models. Docking of the ligands was performed by optimizing attractive interactions and minimizing repulsive interactions. In addition to the agonists used to define the binding site, structurally different agonists are also accommodated by the binding-site model. Furthermore, the m1 receptor binding-site model is able to reproduce experimentally determined stereoselectivities.

Introduction

Genes for more than 100 G-protein coupled (GPC) receptors, including monoamine, acetylcholine (muscarinic), neuropeptide, and opsine (rhodopsine) receptors have been cloned and expressed.[1-4] However, although the amino acid sequences of these receptors are known, their 3D structures are unknown. Thus, it is not possible to use this wealth of information for direct design of specific drugs. Nevertheless, homology-based modeling of these attractive drug targets, in particular when combined with other modeling approaches, may provide the medicinal chemist with important information.

The 3D structure of bacteriorhodopsin (bR) has been determined by high-resolution electron cryo-microscopy.[5] When incorporated into the halobacterial membrane, bR forms seven membrane spanning helices with interconnecting loops. Although there is virtually no sequence homology[3] (see below), the GPC human opsins are related to bR by having a Schiff base bound retinylidene prosthetic group (11-*cis*-retinal cf. bR's *all-trans*-retinal).[6,7] It should be noted that topology can be much better conserved than sequence. In fact, proteins in which the sequence similarity is insignificant (less than 20%) may have the same topology.[8]

Hydropathy plots of GPC receptors are similar to that of bR. These plots show the presence of seven hydrophobic regions, probably corresponding to transmembrane regions (TMs).[9] Further support for this 3D structural arrangement of GPC receptors is provided by studies with antibodies directed toward the intra- and extracellular loop regions of the β_2-receptor.[10] The putative 3D structural homology between bR and the various members of the GPC receptor super family may be used in homology-based modeling of GPC receptor TMs using bR as a template.

Recently, several 3D models of GPC receptors have been reported.[11-19] However, modeling protocols have not been described in enough detail to allow an unambiguous reproduction of the procedures.[20] Herein, we present a detailed protocol for homology-based modeling of the muscarinic m1 receptor[21-23] based on the use of bR as template. In addition, a binding site for m1 agonists is modeled by use of the homology-based m1 receptor model in conjuncture with an indirect model deduced by Schulman et al.[24] The resulting binding site is defined by nine amino acid residues located on TMs 3-6. The combination of indirect and homology-based approaches is particularly attractive since it utilizes more experimental data than a purely homology-based model and since a binding-site model might be more realistic and general in terms of applicability than indirect models. It is noteworthy that the binding-site model correctly predicts m1 agonist activity for a number of analogues not used in the definition of the indirect model.

Methods

The coordinates of bR were obtained from Henderson.[5] The amino acid sequence of the human m1 muscarinic receptor was obtained from the Swissprot database by use of Genetic Computer Group Sequence Analysis Software Package (version 7.1)[25] resident on a VAX. The sequence was subsequently imported in PIR format into Sybyl 5.5[26] on an ESV Workstation (Evans and Sutherland Computer Corporation).

Receptor modeling was performed using Sybyl 5.5. Energy minimizations were made using the following parameters: a distance-dependent dielectric constant of 4,[27] nonbonded cut-off 9.0,[27] AMBER (kollman all atom or united atom),[28,29] conjugate gradient minimization until

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the RMS energy gradient was less than 0.1 kcal/mol·Å^2 (unless otherwise specified).

Small molecules were constructed in their pharmacophore conformations, as described by Schulman et al.,[24] using PCMODEL.[30] The atomic coordinate files were subsequently imported into Sybyl. Compounds not included in the model of Schulman et al.[24] were energy minimized by using the MMX-89.0[30] or MM2(91)[31] force fields and imported into Sybyl. Input structures for the MM2(91) calculations were constructed by using the molecular modeling program MacMimic 2.1.[32]

Homology-Based Modeling. General Strategy. The putative 3D homology between bR and the m1 receptor was applied as strictly as possible in the modeling since experimental evidence on 3D structures of the GPC receptors is lacking and since de novo 3D modeling of proteins, in particular of those that are membrane bound, is poorly understood.[33] Due to the conformational flexibility of the extra- and intracellular loop regions, we have only attempted to model the TMs of the m1 receptor. In order to obtain a homology-based model of the TMs of the m1 receptor, the following protocol was followed: (i) the m1 receptor sequence was aligned with that of bR, (ii) the backbone of bR[5] was used as a template for the positioning of the TMs of the m1 receptor, and (iii) the side chains were adjusted to adopt likely positions. Steps i–iii are described and discussed in detail below.

(i) Sequence Alignment. Since there is a considerable sequence homology between TMs in the various GPC receptors, the TMs may readily be aligned without the introduction of gaps by using conserved amino acids as starting points.[12] It is more complicated to align the TMs of a GPC receptor such as the m1 receptor with those of bR since there is very low sequence homology. A strict TM alignment not only determines the start and end of each TM in the membrane but also the rotation of each TM in relation to the six other (see below). We have considered the following factors in the sequence alignment.

Hydropathy Plots. Tentative identifications of the TMs of the m1 receptor were based on hydropathy plots,[9] i.e., the most hydrophobic parts of the sequence should constitute TMs, but this procedure did not unambiguously identify the beginning and the end of each TM.

Hydrophobicity. The GPC receptor TMs are located in a lipid membrane. The TMs are amphiphilic and should have the hydrophobic face located on the outside, interacting with the lipid layer. Similarly, the polar face of each TM should be located at the relatively hydrophilic interior of the TM bundle. We calculated helical hydrophobic moment vectors[34] of the TMs using the Kyte–Doolittle hydropathy index[9] and used these to aid in obtaining a correct rotation of the helices (these vectors should point toward the outside of the TM bundle; see Figure 1). In addition, prolines were placed on the solvent-exposed face of the TMs (in this case the inside) since this appears to be their preferred position.[35]

Conserved Amino Acids. A number of amino acids in the putative TMs are conserved in a large number of GPC receptors and probably are of functional or structural importance.[36,37] The existence and function of conserved residues are best explained if they are situated on the inside of the TM bundle or in an area which is facing other helices. Residues facing the membrane environment can mutate more easily without affecting the function of the receptor and should be less conserved.[38] Consequently, alignments which position conserved amino acids in a membrane environment were avoided.

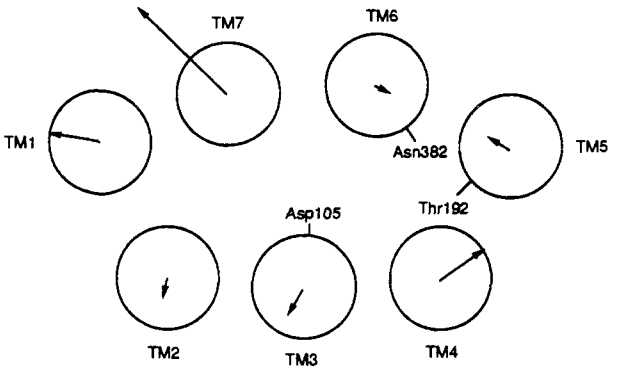


Figure 1. Schematic representation of the transmembrane regions of the homology-based muscarinic m1 receptor model (top view). Hydrophobic moment vectors calculated using the Kyte–Doolittle hydropathy index are shown. Also indicated are the amino acids residues identified to bind agonists in the binding-site model.

Loops. The m1 receptor has a relatively short loop between TM 6 and TM 7 and also between TM 4 and TM 5. This was helpful in determining the exact position of the TMs connected to these loops. Further useful information was provided by a study in which specific antibodies were directed against parts of the loops of the β_2-receptor.[10] The results confirmed the position of some TMs of the β_2-receptor and, by analogy, helped in the definition of TMs of the m1 receptor.

Site-Directed Mutagenesis and Chemical Modification Studies. An important source of information comes from site-directed mutagenesis studies of muscarinic receptors. In a number of studies, residues which are important for the binding of ligands or for the activation of the receptor have been mutated: A mutation of Asp71 to Asn produced mutant receptors with high affinity for carbachol. However, the agonist potency and efficacy of carbachol were lowered at the mutated receptor suggesting that Asp71 is involved in agonist-induced receptor activation.[39] In addition, the equivalents of Asp71 in the α2-adrenergic and the dopamine D2 receptors has been proposed as a putative sodium binding site.[40,41] Substitution of Asp105 with an asparagine produced a receptor with a marked decrease in binding of the muscarinic antagonist [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB). The Asn105 mutant also showed much lower coupling efficiency than the wild-type m1 receptor.[39,41] The Asp105 residue is conserved among all G-protein-coupled receptors with an endogenous ligand containing a basic or quaternized nitrogen and is generally regarded as the binding site for a charged nitrogen. This is further corroborated by chemical modifications of muscarinic receptors with trimethyloxonium ion (TMO$^+$) and propylbenzilylcholine mustard, studies which show that muscarinic ligands bind to a carboxylate-containing side chain in the TM region, most probably Asp105.[42–44]

Muscarinic agonists display low selectivity versus the m1–m5 receptors.[45] In addition, muscarinic receptors are highly homologous, and mutated residues are conserved among the m1 and m3 receptors. Consequently, the following information from site-directed mutagenesis studies on the m3 receptor was useful also in modeling the m1 receptor: Substitution of Thr234 in the m3 receptor by alanine reduced agonist binding affinity 30–40-fold and the maximal stimulation of phosphoinositide (PI) hy-

TM 1	bR	8	PEWIWLALGTALMGLGTLYFLVKGM 32
	m1	27	FIGITTGLLSLATVTGNLLVLISFK 51
TM 2	bR	38	DAKKFYAITTLVPAIAFTMYLSMLL 62
	m1	63	FLLSLACADLIIGTFSMNLYTTYLL 87
TM 3	bR	79	YWARYADWLFTTPLLLLDLALL 100
	m1	98	CDLWLALDYVASNASVMNLLLI 119
TM 4	bR	106	GTILAIVGADGIMIGTGLVGAL 127
	m1	144	MIGLAWLVSFVLWAPAILFWQY 165
TM 5	bR	137	WWAISTAAMLYILYVLFFGFT 157
	m1	188	ITFGTAMAAFYLPVTVMCTLY 208
TM 6	bR	169	STFKVLRNVTVVLWSAYPVVWLI 191
	m1	365	RTLSAILLAFILTWTPYNIMVLV 387
TM 7	bR	202	NIETLLFMVLDVSAKVGFGLILLR 225
	m1	394	CVPETLWELGYWLCYVNSTINPMC 417

Figure 2. Sequence alignment of the transmembrane regions of bacteriorhodopsin and the m1 receptor.

drolysis to 50% of that observed for the wild-type m3 receptor without affecting the binding of antagonists.[46] The Thr234 in the m3 receptor corresponds to Thr192 in the m1 receptor. A mutation of Tyr506 in the m3 receptor to a phenylalanine reduced affinity of agonists 30–40-fold without changing antagonist binding.[46] The corresponding amino acid position in the m1 receptor is Tyr381.

Most probably, the binding of small endogeneous ligands as well as that of agonists takes place on the inside of the TM bundle, and residues that influence the affinity or the efficacy of the ligands should be located in this area of the receptor. Consequently, alignments of the m1 receptor amino acid sequence with bR was made so that Asp79 (TM 2), Asp105 (TM 3), Thr192 (TM 5), and Tyr381 (TM 6) were facing the center of the TM bundle.

There are many possible sequence alignments between the bR and the m1 receptor. We did build models from several different sequence alignments but rejected all but one alignment after having considered the factors presented above. These factors were given different weights in the modeling of the m1 receptor. Studies in which site-directed mutagenesis or alkylation studies have provided clear-cut results were given most weight. The final sequence alignment of the m1 receptor and bR (Figure 2) is similar but not identical to previously proposed alignments.[12]

(ii) Backbone Construction. Helices corresponding to TM 1–7 of the m1 receptor were constructed as right-handed α-helices with $\phi = -55.02°$, $\psi = -50.43°$, and $\omega = 180°$ (mean values from bR). Side-chain conformations shown to be favorable according to protein crystallographic data were used in the helix construction.[47] Proline-containing helices corresponding to TM 4–7 of the m1 receptor constituted a problem. Proline residues have been considered to break helices, but nevertheless, a number of helices contain a proline residue, e.g., bR has prolines in TM 2, TM 3, and TM 6. Proline-containing helices are kinked due to the lack of hydrogen-bonding donor capacity of proline.[48,49] Since the proline residues are conserved among the GPC receptors they may have an important structural or dynamic function,[50] and in our opinion, proline-induced kinks should be included in the modeling.

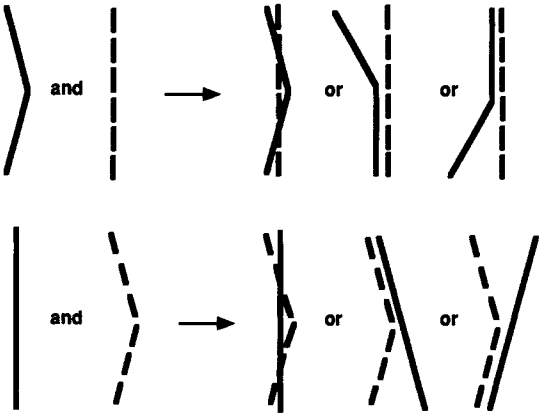


Figure 3. Three extreme superpositions of a kinked (proline containing) helix onto a nonkinked (top) or a nonkinked helix onto a kinked (bottom): (a) a fit involving the complete backbones, (b) a fit involving the part below the kink, and (c) a fit involving the part below the kink.

The positions of the prolines in the GPC receptors and bR are not conserved. Therefore, the kinked helices in bR cannot be used directly as templates for the proline-containing TM 4–7 of the m1 receptor. Further, building and energy minimization of a nonkinked proline-containing helix gives rise to only a slight kink in the helix backbone. Instead, these helices were constructed with a kink typical of a proline-containing α helix.[51]

(iii) Modeling the m1 Receptor TM Bundle. In each of the seven helices corresponding to TM 1–7 of the m1 receptor, side chains were automatically rotated to avoid van der Waals overlap and subsequently energy minimized by use of the Amber force field (all-atom force field).[52] These helices were then combined to form the TM bundle of the m1 receptor by use of TM 1–7 of bR as a template. This procedure is not trivial since it involves superposition of kinked and nonkinked helices. In fact, an infinite number of superpositions are possible, three extremes of which are shown in Figure 3: (a) a fit involving the complete backbone, (b) a fit involving only the part below the kink, or (c) a fit involving that above the kink. We chose to use alternative a because the other two possibilities gave rise to either unacceptable van der Waals overlap between TMs or to large openings in the receptor wall.

As the result of the fitting procedure, van der Waals overlap occurred between side chains of neighboring TMs. These side-chain repulsions were relieved by automatic and manual rotations of dihedral angles within interacting residues while keeping the backbone fixed. Finally, the model of the m1 receptor TM bundle thus obtained was energy minimized (Amber force field-united atoms) with the backbone fixed. Further minimization with no restriction of the helix bundle (2500 iterations) was made (Figure 4).[53] These minimizations did not considerably change the relative positions of the TM 1–7 (mean distance between fitted atoms (backbone) is 0.56 Å), but the aromatic side chains on the outside of the TM bundle markedly changed their conformations.

The resulting homology-based receptor model (Figure 4) has several associative interactions between TMs. In the model there are hydrogen bonds between a number of side chains and between side chains and the backbone.[54] In addition there are interactions between Tyr106, Trp150, Trp157, and Phe154 which are of the favorable edge-to-face type.[55,56]

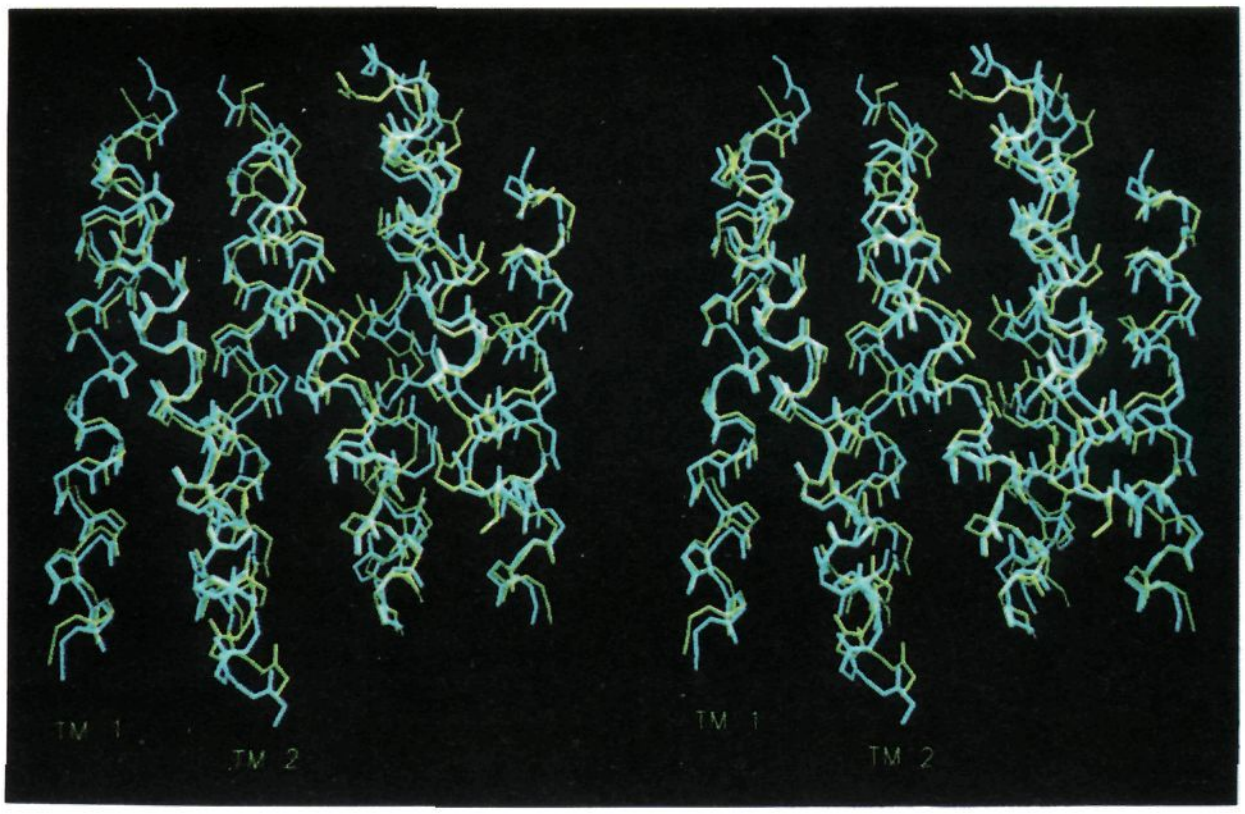
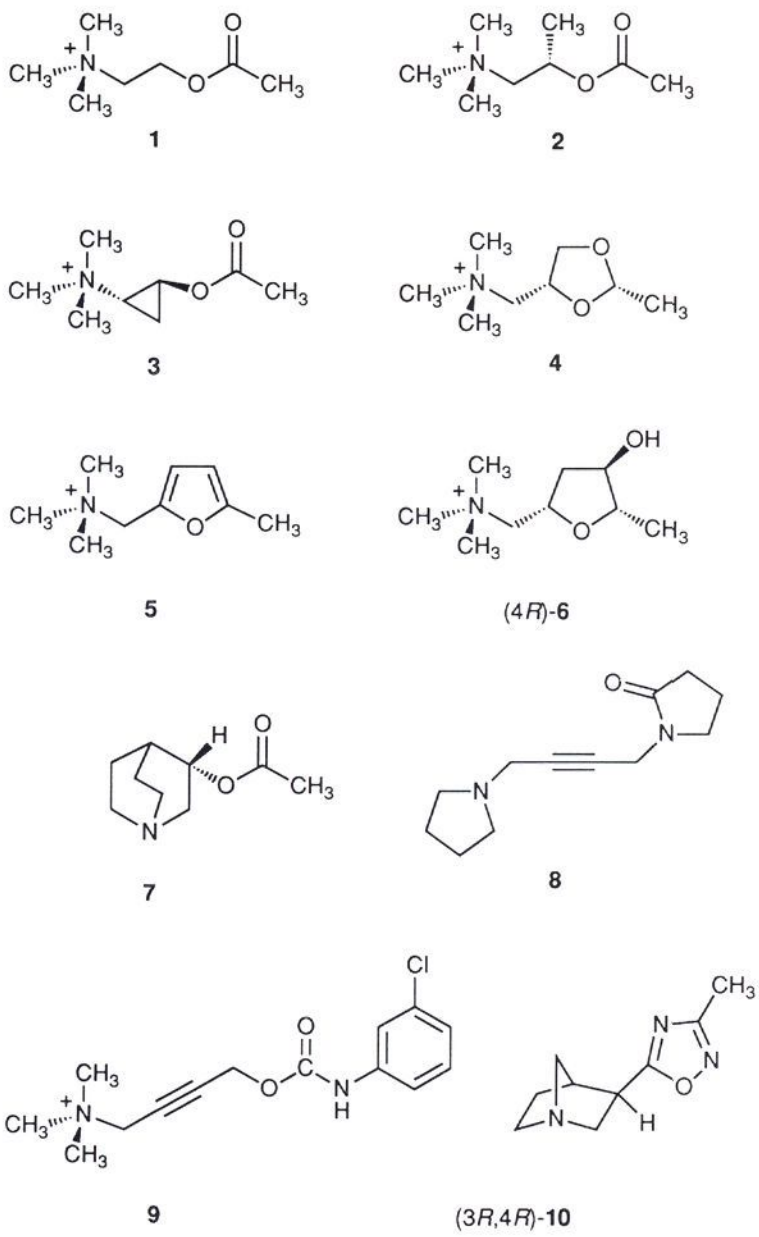


Figure 4. A stereoscopic representation showing a fit of all backbone atoms of the transmembrane regions of bacteriorhodopsin (cyan) with those of the energy-minimized m1 receptor model (yellow). The average distance between fitted atoms is 1.20 Å.

Chart I. Structures of the Muscarinic Agonists Considered in the Model



An Indirect Model for m1 Receptor Agonists. On the basis of a detailed conformational analysis using molecular mechanics and ab initio calculations of a series of muscarinic agonists (see Chart I) containing a NCCOCC backbone, Schulman and co-workers have developed an interesting pharmacophore model (Figure 5).[24,57–59] Interactions were believed to exist between the cationic head group of the ligands and an anionic receptor site and

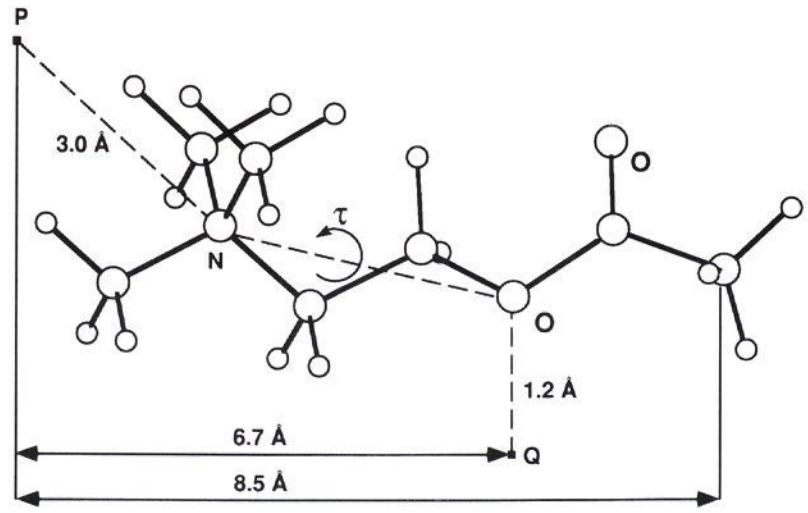


Figure 5. A representation of the model of the active conformation of muscarinic agonists presented by Schulman et al.[24] as exemplified by acetylcholine. The dummy atoms P and Q were added 3.0 and 1.2 Å from the nitrogen and the ether oxgyen, respectively, to represent interaction points with the receptor. The distance between P and Q and P and the acyl methyl group is relatively constant. In muscarinic agonists, the dihedral angle τ (PNOQ) may adopt values between 100 and 117°.

between an ether oxygen of the ligand and an attractive entity on the receptor (this latter interaction might be a hydrogen bond). The model defines two interaction sites P and Q located 3.0 and 1.2 Å from the nitrogen and ether oxgyen, respectively. The "interaction dihedral angle" between these four points (PNOQ) was determined to be 100–117° in pharmacophore conformations of the agonists. Since P and Q correspond to atoms or structural units at the receptor, the distance between them should be relatively fixed and was not estimated to vary more than 0.3 Å between different agonists. In addition, the acyl methyl group is of importance of activity[60] and should be located in a relatively fixed position.

To derive a receptor-excluded volume[58,59] from the above pharmacophore model, we constructed a set of molecules used by Schulman et al.[24] (**1-5**, Chart I) in their pharmacophore conformations and dummy atoms were added: 3.0 Å from the nitrogen (P) and 1.2 Å from the ether oxygen in the COC plane (Q). The agonists were superimposed by least-square fits of the dummy atoms and the acyl methyl group, using acetylcholine (**1**) as the template. In

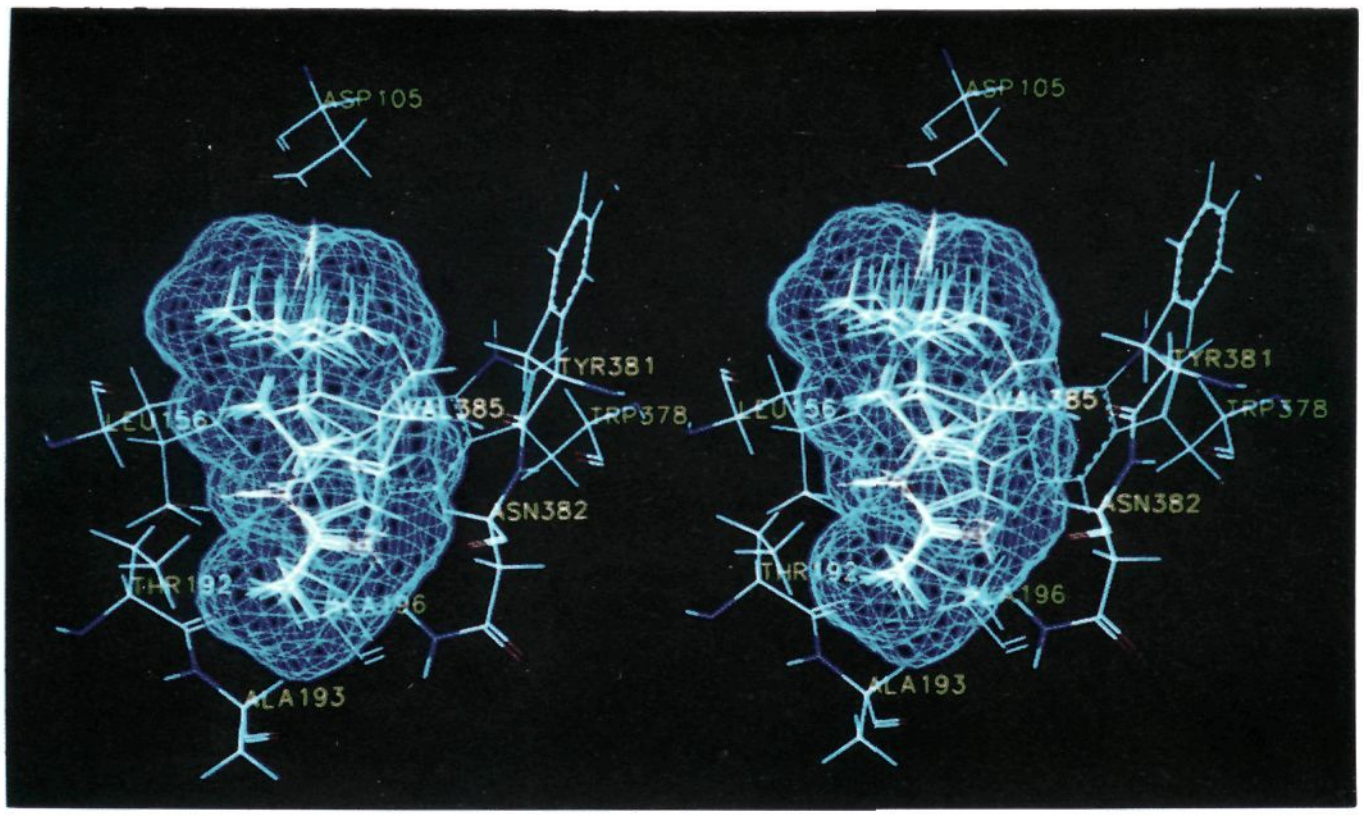


Figure 6. Stereoview of the m1 receptor excluded volume fitted into the (adjusted) putative agonist binding site of the m1 receptor.

terms of the active analogue approach,[58,59] the combined van der Waals volume of the agonists (Figure 6) constitutes a muscarinic receptor-excluded volume. Since the compounds used by Schulman are potent agonists at the m1 receptor,[61-63] the volume generated also represents a m1 receptor-excluded volume.

Binding-Site Modeling. The homology-based model of the m1 receptor described above represents one of an infinite number of possible models. Consequently, the relevance of homology-based receptor models can only be assessed by evaluation of their ability to accommodate experimental data. Such a test may be performed by docking a series of pharmacologically well-defined ligands (which should fit well) and inactive analogues (which should not fit at all in energetically accessible conformations and/or should produce steric overlap with the receptor). However, docking of a flexible molecule like 1 with many available low-energy conformations into the homology-based model of the m1 receptor generates several possible conformations of the ligand which may appear to bind well. In addition, one has to take into account the flexibility of the amino acid residues interacting with the ligand.

To simplify the evaluation procedure and to better define the binding site, we used the m1 receptor-excluded volume derived from Schulman's model to probe a common binding site for agonists. The docking procedure involved manual docking of the receptor-excluded volume into the homology-based m1 receptor model using one of the oxygens of the carboxylate at Asp105 as an anchoring point. Attractive interactions were optimized, and repulsive interactions were minimized during the docking. In addition, the conformations of the side chains forming the putative binding site were changed manually to minimize overlap between the homology-based m1 receptor model and the m1 receptor-excluded volume. The binding site is fully defined by the following nine amino acids: Asp105 (TM 3), Leu156 (TM 4), Thr192, Ala193, Ala196 (TM 5), Trp378, Tyr381, Asn382, Val385 (TM 6) (for coordinates, see the supplementary material).

There are a number of attractive interactions between the agonist ligands and the amino acid residues in the model of the binding site; e.g., interactions with 1 occur from Asp105 to the positively charged ammonium group, from Thr192 to the ether oxygen, and from Asn382 to the carbonyl oxygen (Figure 7). There is an attractive van der Waals interaction between the methyl group of 1 and the methyl group in Thr192. The charged ammonium group is located close to Asp105, Trp378, and Tyr381. Considerable evidence has accumulated which indicates that aromatic side chains (especially from the more electron-rich residues tyrosine and tryptophane) can interact with trimethylammonium groups through favorable cation-π interactions.[64,65] Most likely, the interaction between the cationic ligand and the carboxylate is therefore further stabilized by these aromatic residues. In fact, the aromatic residues located "above" Asp105 (Trp101, Phe163, Trp164, Tyr381, and Trp400) may facilitate the entry of the ligands into the TM bundle (an "aromatic gorge").[66] In addition, hydrogen bonds may form between 1 and Thr192 and Asn382, respectively. These bonds are of reasonable length and have acceptable angles (Table I).[67-70]

Some of the criticisms of the model of Schulman et al. were related to its simplicity:[57] it only takes into account one interaction between the ester group of 1 or its equivalent and the receptor. The present m1 binding-site model demonstrates that it is possible to extend the model of Schulman et al. to a model that has two attractive interactions with the ester moiety.

Evaluation of the Binding-Site Model. The conformations and relative orientations of the ligands in indirect models, such as the one developed by Schulman et al., may be regarded as mean values. An indirect model describes the best average binding mode for a number of ligands. However, the flexibility of the side chains of the receptor/enzyme is not considered. During the binding of the ligand to the receptor, the side chains defining the binding site may change their conformations to better accommodate the ligand into the receptor and to maximize attractive interactions. Therefore we have also docked every ligand individually with the binding site to try to obtain an optimal complementarity of each ligand and the receptor cavity. Pharmacophore conformations of the ligands were used, and torsion angles in side chains forming the binding site were rotated to minimize repulsive and maximize attractive interactions. Figure 8 shows the

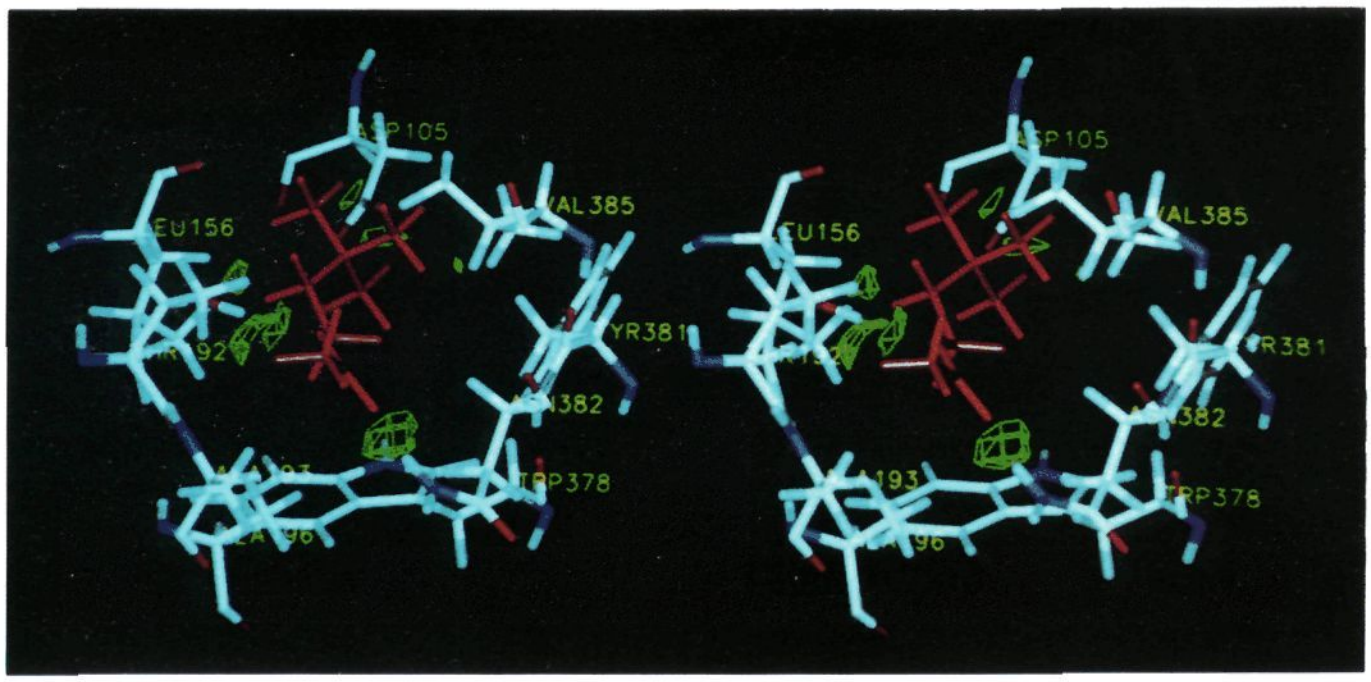


Figure 7. Stereo representation of the interaction of **1** (red) with the m1 binding-site model. Green areas represent van der Waals volume overlap between ligand and receptor.

Table I. Binding Distances (Å) and Angles (deg) between Amino Acid Side Chains in the m1 Receptor Binding-Site Model and the Docked Ligands

	Asp105[a]		Thr192		Asn382		volume overlap	
compound	distance	angle	distance[b]	angle[c]	distance[d]	angle[e]	V^f	% V^g
1	3.58		2.91	158	2.73	163	3.3	2.24
2	3.63		3.15	157	2.78	152	3.2	1.96
3	3.70		3.02	167	2.84	170	3.7	2.38
4	3.81		3.01	159	2.79	167	3.0	1.86
5	3.84		2.58	169			3.5	2.28
(4*R*)-**6**	3.80		2.96	158	2.84	150	4.6	2.61
(4*S*)-**6**	4.15		2.84	159	2.74	143	7.3	4.21
7	2.87	161	2.63	175			4.1	2.59
8	2.62	169			2.85	163	5.8	2.92
9	3.53				2.72	159	6.5	2.75
(3*R*,4*R*)-**10**	2.79	153	2.89	162	3.06	164	5.4	3.35
(3*S*,4*S*)-**10**	2.71	150	2.77	172	3.00	172	5.7	3.60
(3*R*,4*S*)-**10**	2.96	171	3.0	160	2.88	163	4.6	2.98
(3*S*,4*R*)-**10**	2.74	172	2.69	158	2.89	163	6.5	4.10

[a] The distance between one Asp105 oxygen and the agonist nitrogen. For protonated tertiary amines the angle between the nitrogen, hydrogen, and carboxylic oxygen is also given. [b] The distance between the Thr192 oxygen and the agonist ether oxygen (or equivalent). [c] The angle between the Thr192 oxygen, hydrogen and the agonist ether oxygen (or equivalent). [d] The distance between the Asn382 oxygen and the agonist ether oxygen (or equivalent). [e] The angle between the Asn382 oxygen, hydrogen, and the agonist ether oxygen (or equivalent). [f] The common volume between the binding-site model and the agonist. Given by the Sybyl MVOLUME command. [g] Common volume between binding-site model and the agonist given in percent of the agonist volume.

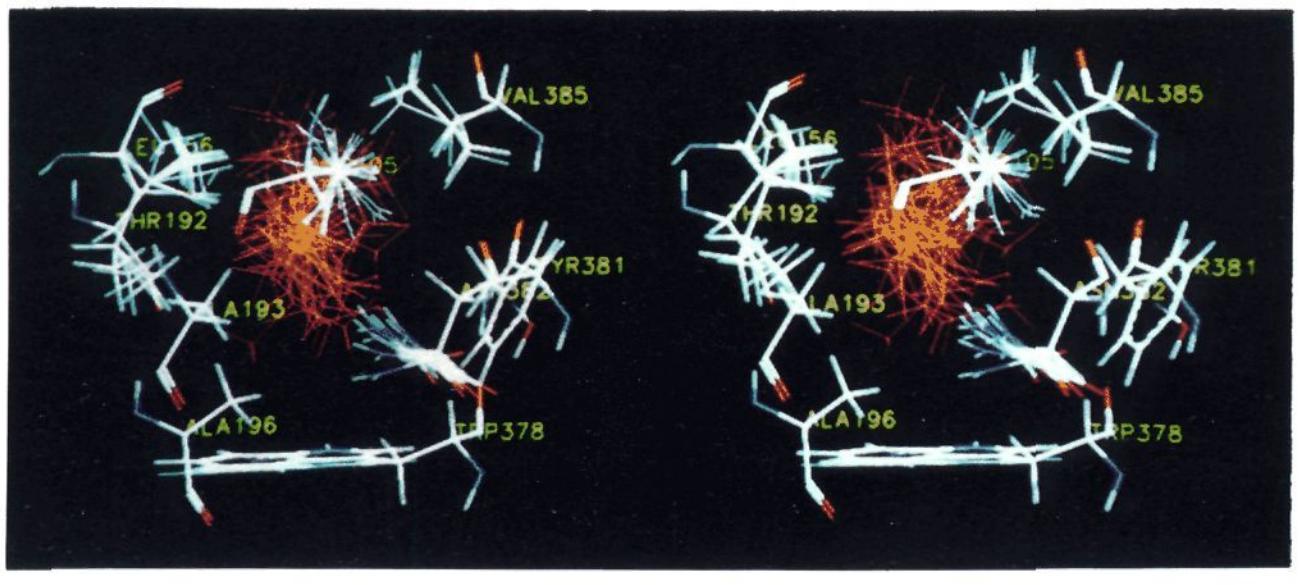


Figure 8. Stereo representation showing the muscarinic agonists in Scheme I (red) individually docked into the putative m1 receptor binding site. The figure illustrates the multiple binding modes that can exist for the ligands as well as the flexibility of the side chains in the binding site.

modes of binding the ligands to the binding site and the different orientations of the side chains.

In addition to the compounds included in the generation of the binding-site model we docked a number of other potent muscarinic agonists. Muscarine ((4*R*)-**6**), although included in the model of Schulman et al., was not part of the receptor excluded volume used for construction of the binding-site model, since we were unable to minimize steric repulsion and to fit the hydroxyl group of muscarine to Asn382 while retaining a good fit to corresponding groups

in the other compounds. We therefore fitted (4*R*)-6 alone to our binding-site model while retaining the pharmacophore conformation proposed by Schulman et al. In this way we were able to produce a good fit between (4*R*)-6 and the m1 binding-site model. This illustrates that multiple binding modes might be possible even within a class of relatively similar compounds. Muscarine is highly stereoselective in its actions,[71] and we could reproduce this by docking experiments with the weakly potent 4-hydroxy epimer ((4*S*)-6). Docking of this epimer using the same orientation of the 5-membered ring as for muscarine produced an interaction between the ether oxygen and Thr192. The hydrogen bond between the hydroxyl group and Asn382 found in (4*R*)-6 is broken in (4*S*)-6 due to the change in stereochemistry (Figure 9). Therefore, (4*S*)-6 was fitted into the binding site in a different fashion with the ether oxygen and the hydroxyl group interacting with Thr192 and Asn383 in the same way as (4*R*)-6. In this mode of binding, (4*S*)-6 produces a considerably larger van der Waals overlap than the epimer (4*R*)-6. In addition, this mode of docking produces a distance between the charged nitrogen and Asp105 which seems to be too long. Several additional conformations of (4*S*)-6 were docked into the binding site without further improvement of binding distances, angles, or van der Waals overlap. The inactivity of (4*S*)-6 may therefore be explained by the loss of one point of interaction between receptor and ligand and/or the less good fit of the ligand into the binding site indicated by the increased van der Waals overlap. These observations corroborate the binding-site model.

The classical muscarinic agonist oxotremorine (8) is well accommodated by the binding-site model.[72] It binds in a transoid conformation. Attractive interactions are possible between the Asn382 and the carbonyl of 8 and the protonated nitrogen and Asp105. Compound 9 (McN-A-343) is an M_1-selective muscarinic agonist.[72] This compound has more steric bulk than other agonists due to its phenyl substituent. Therefore, when docking 9 into the binding-site model, positions of side chains of some residues outside the binding site were slightly modified (by rotating around single bonds). Interactions were observed between the carbonyl oxygen and Asn382 and between the trimethylammonium group and Asp105.

We have also studied the interaction between the potent muscarinic agonist (3*R*,4*R*)-3-(3-methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane (10) and the m1 receptor binding-site model.[73] Compound 10 is well accommodated by the model[74] with hydrogen bonds between the oxadiazole nitrogens and the Thr192 and Asn382, respectively, and a reinforced ionic bond[75] between the protonated nitrogen and the carboxylate as proposed by Saunders et al. (Figure 9).[73,76] We were also able to dock the three other stereoisomers of 10 into the binding-site model. Although all isomers are agonists, there is a 100-fold difference between the most potent isomer and its diastereomers and a 10-fold difference to its enantiomer. These differences may be rationalized in qualitative terms by taking into account differences in the common van der Waals volume between the ligand and the binding site and/or in the relative energy of the receptor-bound conformation. For instance, (3*R*,4*S*)-10 produces small steric overlap, but the bound conformation has an energy 2.0 kcal/mol above the minimum-energy conformation. In contrast, (3*S*,4*R*)-10 interacts with the model in the minimum-energy conformation but produces a larger steric overlap.[77]

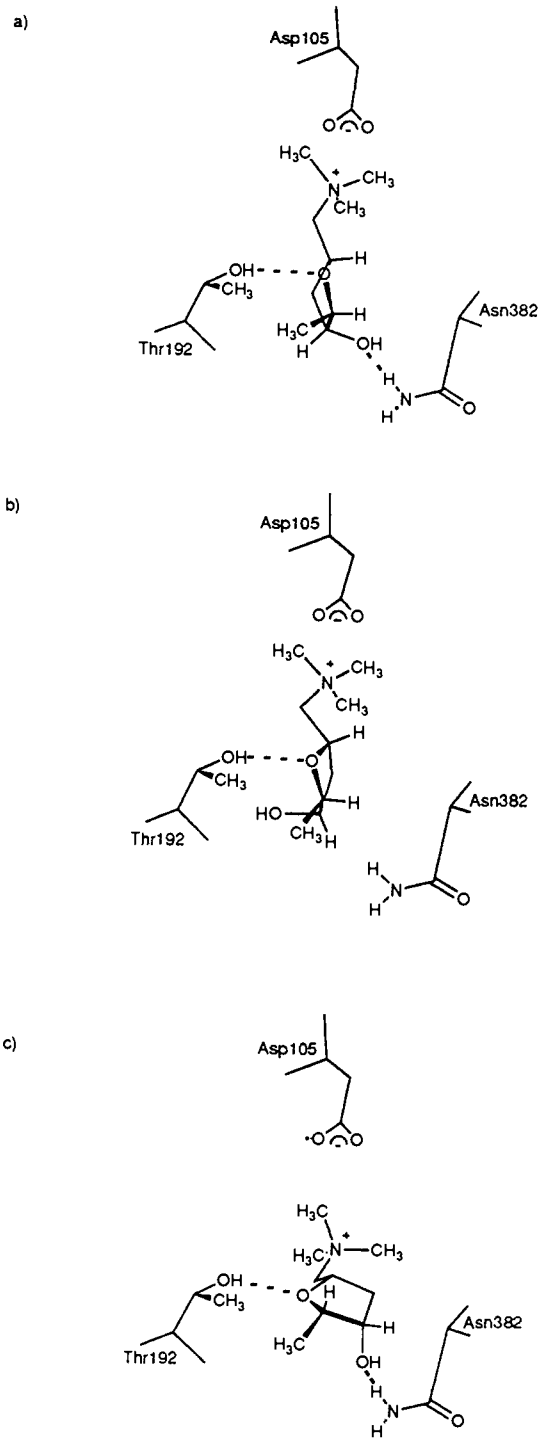


Figure 9. Schematic representation of the different binding modes of (4*R*)-6 and (4*S*)-6: (a) (4*R*)-6 has three points of interaction with the binding site. (b) The inverted stereochemistry at C4 results in loss of one interaction point if (4*S*)-6 is placed with the same relative ring orientation as the more potent (4*R*)-6. (c) If (4*S*)-6 is positioned to accept two hydrogen bonds, the van der Waals overlap with the receptor is considerably larger than that of (4*R*)-6. In addition the distance from the nitrogen to the carboxylate is longer for (4*S*)-6 than for (4*R*)-6 (see also Table I).

Conclusions

The m1 receptor binding-site model described herein was obtained by a combination of homology-based mod-

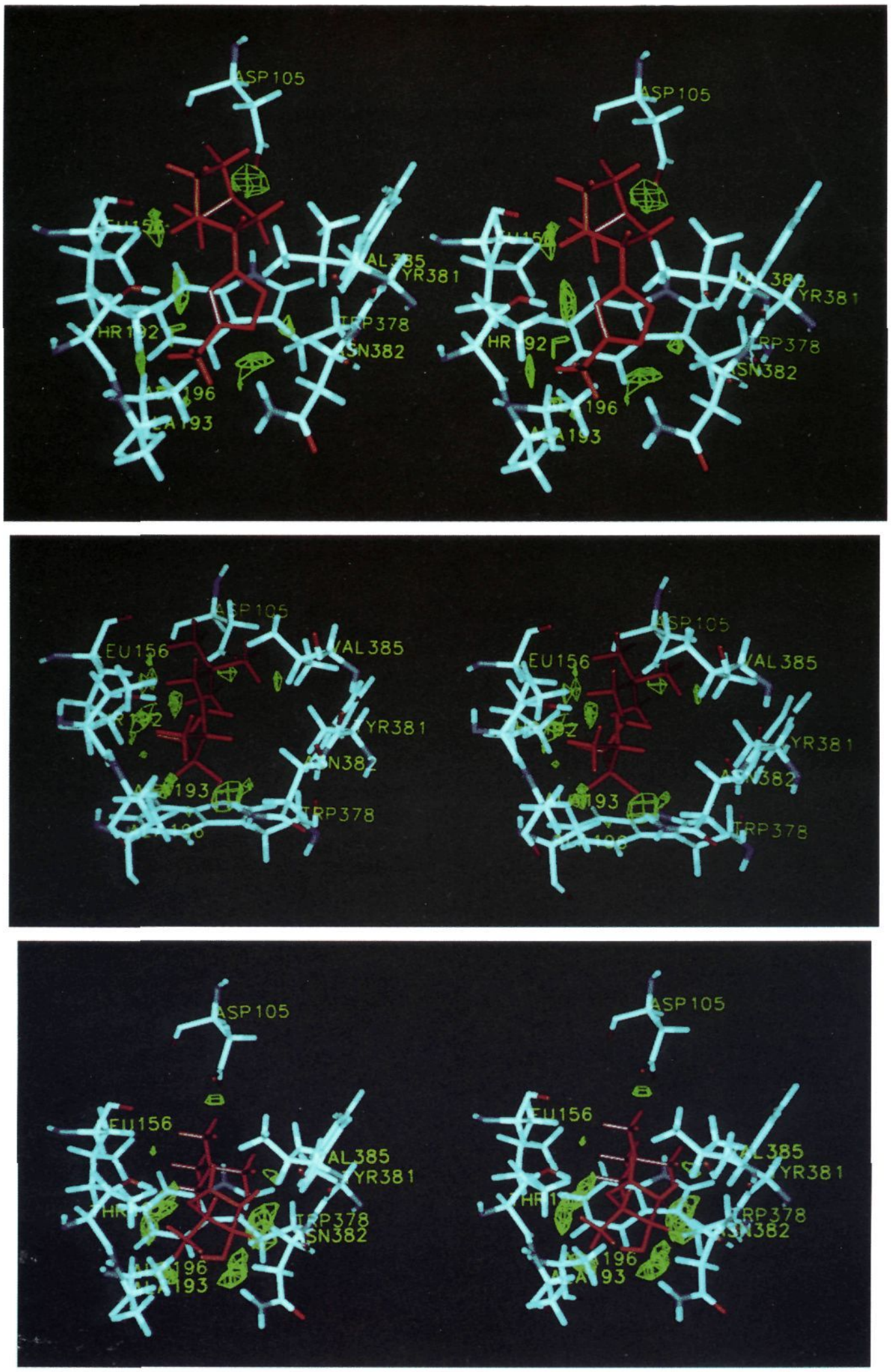


Figure 10. Dockings of the agonists (3*R*,4*R*)-**10** (top) and muscarine ((4*R*)-**6**; middle) into the m1 receptor binding-site model. At the bottom, the unsatisfactory docking of the inactive 4*S*-muscarine (4*S*-**6**) is shown (see also Figure 9). Green areas represent van der Waals volume overlap between ligand and receptor.

eling and indirect modeling based on a series of agonists. This combination (a) provides a maximal input of experimental data into the modeling process, (b) may be applied to binding-site modeling of other GPC receptors, and (c) provides potential basis for modeling of the signal transduction process which leads to activation of the G-proteins.

The m1 receptor binding-site model should be of general applicability in the design of novel agonists since it is able to accommodate agonists which are structurally different from those used in the generation of the model and since it is able to differentiate between active and less active/inactive stereoisomers. The model is dynamic in that the nine amino acids which define the binding site may adopt various conformations to allow for an optimal complementarity between agonist and binding site. In our opinion, a dynamic model is more realistic than the static

models generated in the active analogue approach and other indirect modeling techniques.

The problem of multiple minima[80] has discouraged us from calculating energies for the interaction between the binding-site model and various agonists. Thus, we consider the model to be primarily of qualitative value. However, one may foresee applications in which 3D QSAR (CoMFA)[81] is used in conjuncture with homology modeling to produce refined quantitative models for limited sets of compounds which bind similarly or identically to the receptor.

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Supplementary Material Available: Table of atomic coordinates for the residues defining the m1 receptor binding-site model (3 pages). Ordering information is given on any current masthead page.

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