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The High Affinity Melatonin Binding Site Probed with Conformationally Restricted Ligands—II. Homology Modeling of the Receptor

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Abstract—We present the first 3-D model of the melatonin receptor based on the recently published amino acid sequence of the cloned melatonin receptor. The seven trans membrane helices were positioned using the helices found in the structure of BacterioRhodopsine. From the results of an indirect modeling study with six melatonergic agents, an alignment of these compounds was found directing towards common interaction points. These points are suggested to be the two serines in helix three and the histidine in helix five, forming hydrogen bonds with the amide function and the methoxy-oxygen in melatonin, respectively. The ligands were docked into these binding sites and the receptor–ligand complexes were energy minimized. Considering the position of the active and inactive ligands in the receptor and their respective occupied volumes, the structure–activity relationships are rationalized by the suggested model. This model can be of use as a pharmacological test model in molecular biological studies and as a basis to develop compounds being active as synchronizing circadian agents. Copyright © 1996 Elsevier Science Ltd

Introduction

Since melatonin was isolated and identified as *N*-acetyl-5-methoxytryptamine by Lerner in 1958,[1] the interest in this hormone has been steadily growing. In vertebrates this hormone has its primary sites of production in the pineal gland and the photoreceptor cells of the retina, where it is synthesized from serotonin via a two-step biochemical pathway.[2,3]

Melatonin plays a fundamental role in translating photoperiodic information and modulates a variety of endocrinological, neurophysiological and behavioral functions in vertebrates.[4–7]

The exact function of melatonin in man is not fully elucidated although the presence of melatonin receptors are demonstrated in the human circadian clock [8].

Its potential usefulness to a number of therapeutic areas is recognized: it may find application in a synchronization of disturbed circadian rhythms such as jet-lag ,[9] sleep–wake cycles,[10] seasonal disorders,[11] and depression.[12] Perhaps more contentiously, even a role in degenerative processes is suggested.[13]

All of the studies were pharmacologically and physiologically oriented and based on the melatonin molecule itself. Structure–activity studies were rare and limited to simple derivatives of melatonin.[14,15] Only more recently analogues of melatonin were designed and synthesized as the naphthalenic bioisosteres[16] **2** and the conformationally restricted analogues of melatonin like carbazole derivatives [17] **4** and **5**, the amidotetralin derivatives **3**, and the amidoindan **6**.[18]

It can be expected that the interest in the melatonergic system and the compounds that can effect this system will be growing considering the potential therapeutic uses.[19] Not least, this will be stimulated by the publication of the primary amino acid sequence of a cloned melatonin receptor.[20] Almost certainly, subtypes of this receptor will be discovered, as is seen in other drug areas, that might be the start for the synthesis of specific drugs for these subtypes.

In order to stimulate the design of novel drugs and to interpret the activity data of the known compounds, we present here the construction of a three-dimensional model for the melatonin receptor and propose an interaction place for melatonin and some of its synthetic analogues.

Modeling

The synthesis of ^{125}I 2-iodomelatonin as a high affinity radiolabel[21] for melatonin receptors and the recent development of quantitative in vitro bioassays[22–24] has enabled the screening of melatonin analogues for their affinity and pharmacological characteristics. The high affinity binding of agonists could be modulated by guanine nucleotides, indicating coupling to a G-protein,[25–27] which was substantiated by the finding that melatonin was able to inhibit the forskolin stimulation of cAMP.[28]

The assumption that the melatonin receptor belongs to the family of G-protein-coupled receptors was strongly supported when the melatonin receptor was cloned using a cDNA library constructed from a cell line of Xenopus dermal melanophores.[20]

The protein that was encoded by the cDNA in COS-7 cells had a high affinity iodomelatonin binding, that could be inhibited by six ligands in a rank order that was identical to that reported for endogenous high affinity receptors. Functional studies of CHO cells showed that melatonin acting through the cloned receptor inhibited forskolin-stimulated cAMP accumulation in a dose-dependent manner.

Hydropathy analysis of the amino acid sequence revealed the presence of seven hydrophobic segments, which likely represent the transmembrane regions of a G-protein receptor (GPC receptor)

Recently the human melatonin receptor was cloned,[29] being 77% identical within the trans membrane domains to that of the Xenopus melatonin receptor.

The acquired knowledge over the last few years on the amino acid sequences of an array of GPC receptors and the availability of a high-resolution structure of BacterioRhodopsine [30] has been the basis for the prediction of the 3-D structure for GPC receptors.[31,32]

These models have been derived to a greater or lesser extent from the structure of BacterioRhodopsine, although the sequence homology of the GPC with the BacR is poor (6–11%).[33] The modeling studies varied from the pure theoretical approach of constructing ideal helices,[34,35] through the construction of energy-minimized helices and fitting them onto the backbone of the BacR helices,[33,36–39] to the use of the coordinates of BacR and substituting the amino acids.[40] As this choice of the general approach is only one of the many choices one has to make in constructing a GPC-receptor model, it is obvious that the final result will be one model out of the thousands possible. A reliable model should be able to explain in a qualitative way the structural features derived from the structure–activity relationships of related agonists/antagonists, and in this way, function as a template on which new compounds can be designed and tested.

Methods

Construction of helices and receptor. Considering the amino acid sequence derived from the hydropathy analysis, we accepted these sequences as a base for constructing the seven transmembrane helices (Fig. 1). These helices were constructed following the general building procedure in Sybyl 6.1 (Tripos Associates Inc., St. Louis, MO) according to the standard angles for an α-helix. The helices were then energy minimized using the Tripos force-field, Gasteiger–Hückel charges, a distance-dependent dielectric constant of 5, and a nonbonded cutoff of 8 Å. The minimization was performed using the conjugated gradient minimization until the rms energy gradient was less then 0.1 kcal/mol Å.

As is generally accepted and shown in BacR the helices tend to position themselves with the nonpolar residues facing the lipid alkyl chains of the membrane.[41] The procedure used in this process is based on the construction of so-called helical wheels,[42] where the helix residues are projected down the helical axis so that the amphipathicity—the separation of nonpolar and polar residues—is apparent. Although it is not always obvious which site of the helix is the most polar one, we rotated the helices considering the amphipathicity and the potential residues involved in binding as a first approach. The backbones of the helices were then placed visually onto the backbone of BacR. With the use of the van der Waals volumes of the helices they were positioned in such a way that steric interaction with the side-chains were minimized. At the end this will result in a receptor in which the several helices are not exactly matching the BacR helices, which is obvious regarding their poor homology. So BacR is used as a rough template to give an indication of how the trans membrane bundles of helices might be positioned in the GPCR family.

After each addition of a helix the complex was energy minimized with the parameters described above.

Indirect modeling models. The minimized structures **1** to **6** (Chart 1) from an indirect modeling study[43] were used as starting conformers. In this study a conformational analysis was made and pharmacophores were

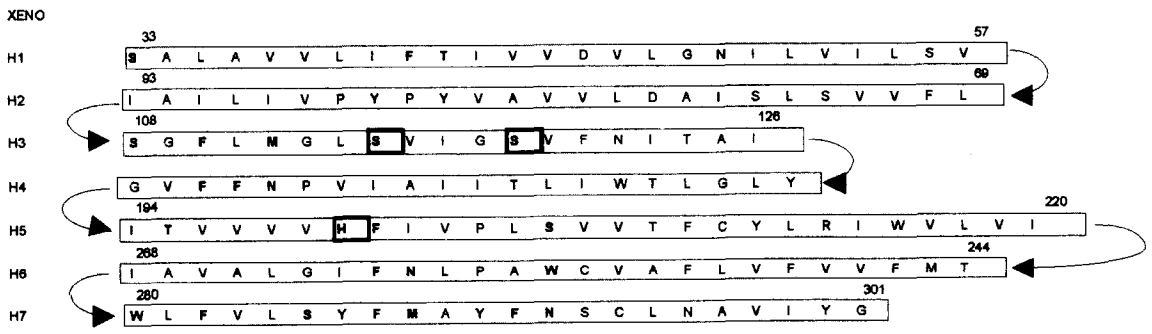

Figure 1. Amino acid sequence. Sequence of the amino acids in the seven transmembrane helices of the melatonin receptor. The helices are displayed from left to right according to the position from the upper part to the cystolic part of the transmembrane bundle. Receptor residues in the upper part capable of forming an interaction are bold.

identified with the APOLLO program, using pharmacophoric points extending from the amide and methoxy functional groups. Around the clusters of superpositioned molecules, minireceptors were build using the Yak program. Based on the observed interactions, one of the pharmacophore models was selected for subsequent use (model B).[43]

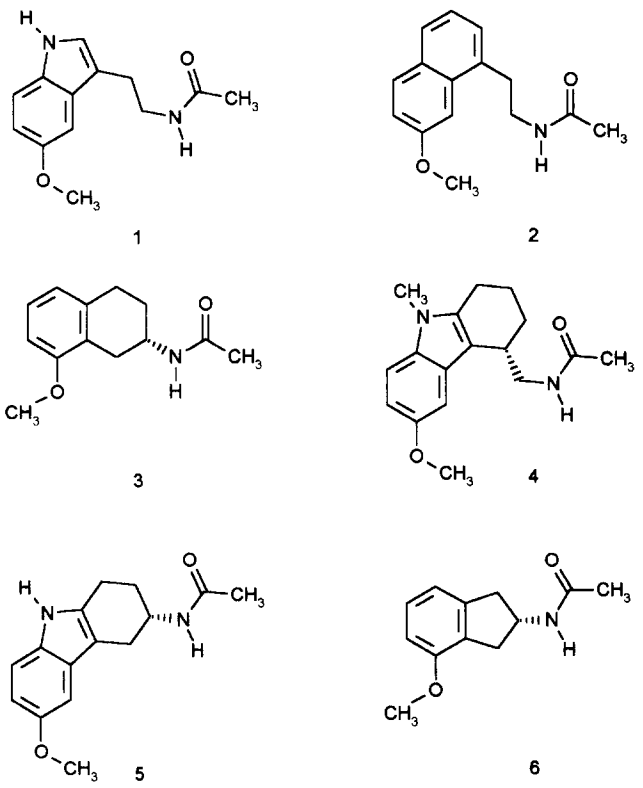

Chart 1.

The amino acids capable of forming hydrogen bonds in the upper part of the helix bundle were used as probes for the melatonin molecule. In this way our attention was directed to the serines in helix three and the histidine in helix five as possible candidates for interaction points. After fine-tuning the position of helices three, four, and five around melatonin, the whole receptor was energy minimized. The position of the helices relative to those in BacR gave a reasonable agreement as shown in Figure 2. A schematic representation of the binding site with adjacent residues and melatonin placed into it is shown in Figure 3.

Modeling of the receptor–ligand complexes. The compounds **1** to **6** from the indirect modeling study were first minimized (rms 0.01 kcal/mol Å) with the parameters used to minimize the receptor. During this procedure, the conformations of the compounds hardly changed from the input structures. This indicates that with the force field we used to minimize the receptor, the starting conformations were close to a local minimum. As it was our intention to keep the ligands as close as possible to this local minimum, we performed the docking procedure in discrete steps, with increasing modification in torsion angles of the receptor and ligands. The compounds were first manually fitted into the binding site region of the receptor in such a way that the amide group interacted with the two serines and the methoxy-O with the histidine-H, forming three hydrogen bonds, indicated by

Figure 2. Melatonin receptor. Top view of the melatonin receptor (green) and the BacterioRhodopsine structure (cyan).

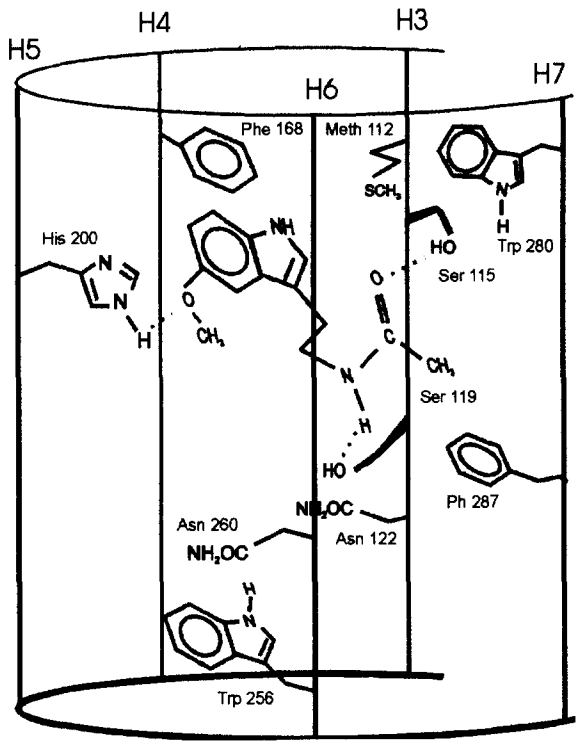

Figure 3. Ligand binding site. Schematic representation of the ligand binding site in the melatonin receptor model. The agonist melatonin is located in the binding site. Interactions occur between methoxy-oxygen and the His-200 and between the amide group and the Ser-115 and Ser-119.

Sybyl as dotted lines, corresponding to a distance of 1.7–2.7 Å. The ligand was manually positioned in the Docking procedure of Sybyl, trying to obtain a minimal steric interaction energy while maintaining the hydrogen bonds. During these processes the torsion angles involved in the serines and the histidine of the binding site were made rotatable and, when necessary, adjusted. When no minimal interaction energy was obtained, still keeping the hydrogen bonds, the methoxy group in the ligand was rotated, resulting in a conformation of the ligand that was a new minimum or a conformation very close to such a minimum.

For melatonin, the naftyl compound and the carbazoles, no changes had to be made to fit these compounds into the receptor. The methoxy group of the tetralin and the indan had to be moved out of their starting positions to minimize steric interactions. The carbazole **4**, being a very rigid compound, could be placed with difficulty into the receptor, partly due to the *N*-indole-methyl substituent.

After these adjustments had taken place, the ligands were MERGED into the receptor. Then the whole complex was energy minimized (rms = 0.1 kcal/mol Å). For most compounds conformational changes took place in the torsion angles for the serines and histidine in the receptor and in flexible angles of the ligands and hydrogen bonds were broken. To restore the hydrogen bonds a repeated DOCKING procedure was performed in which the hydrogen bonds were constrained (Hist-OCH_3 1.5–2.7 Å, Ser-C=O; 1.3–2.7 Å, Ser-NH; 1.5–2.7 Å).

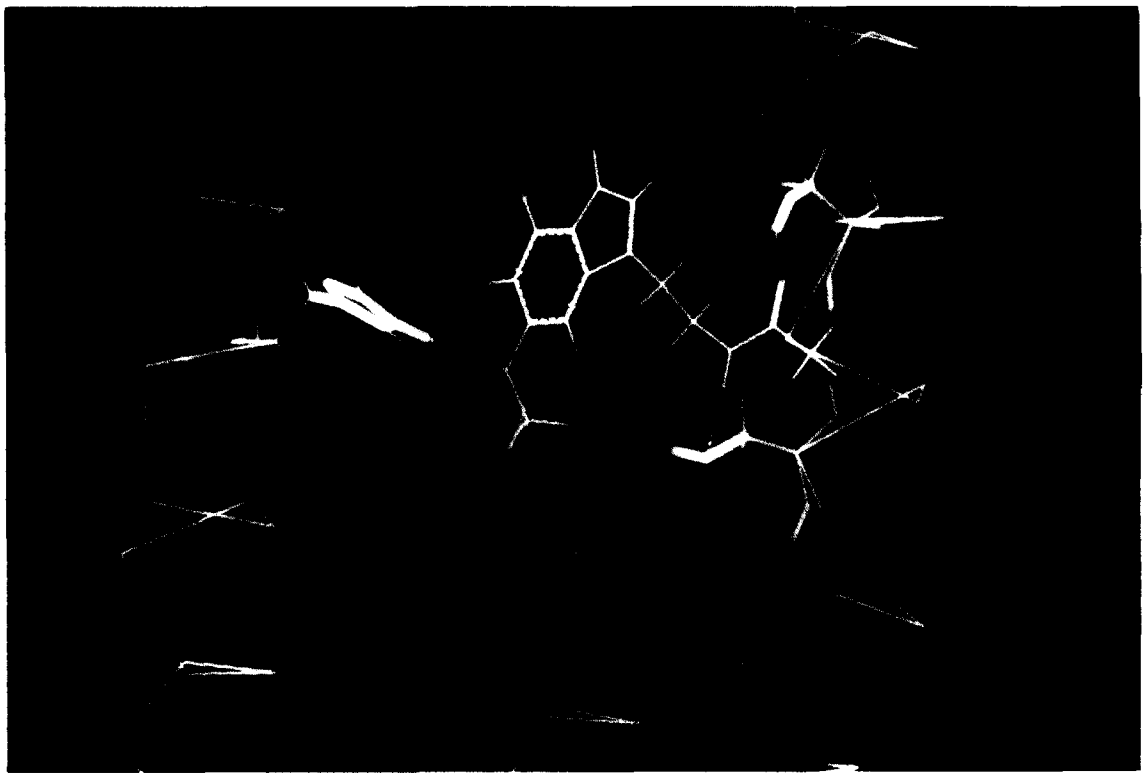

Figure 4. Melatonin in the ligand binding site. Steric representation of the interations between the energy minimized complex of the melatonin molecule in the receptor. Hydrogen bonds are indicated by dotted lines.

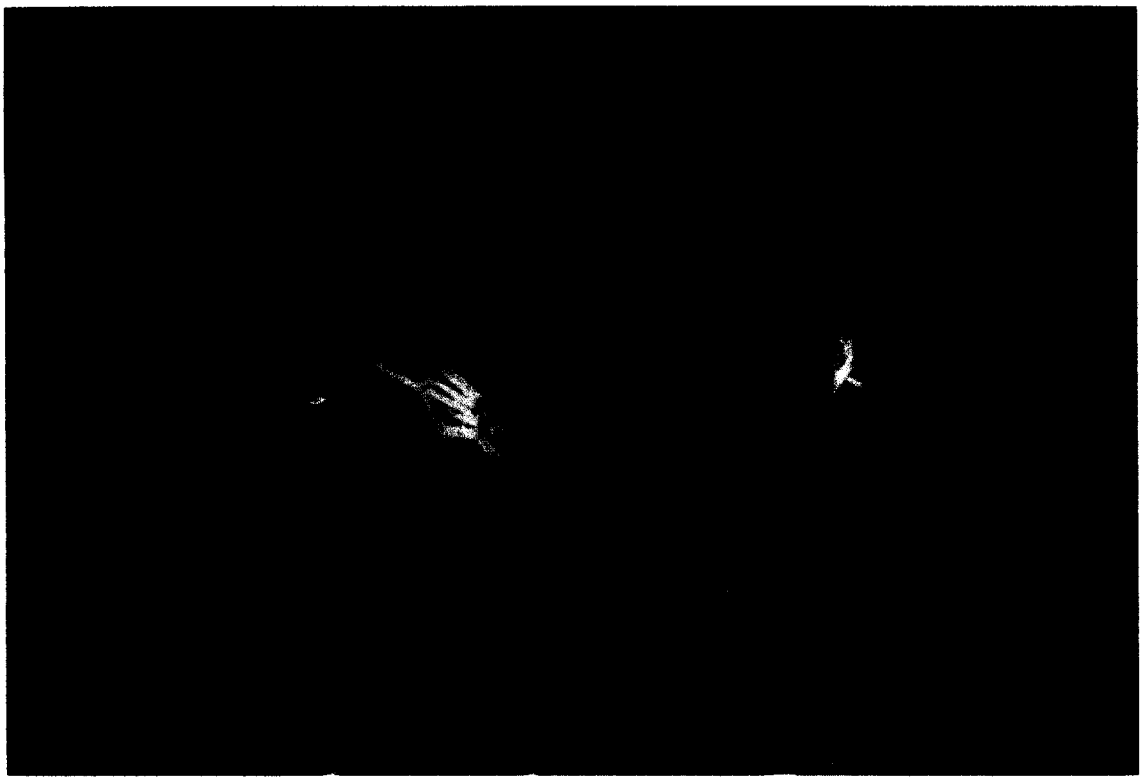

Figure 5. Melatonin agonists. The energy minimized structures of the receptor–ligand complexes with the melatonin agonists **1–4**.

Results and Discussion

In this study a series of melatonergic compounds with different affinities towards the melatonin receptor was used in a direct modeling study to locate the interaction points of these ligands with the receptor.

Until now we did not have any indication which amino acids are important in the binding processes as no point mutations have been applied for the melatonin receptor. From the well-studied neurotransmitter systems, such as the serotonin, the adrenergic, and the dopamine receptors, we know that the interactions are taking place with amino acids in the third and fifth helix. Mutation studies supported the importance of an Asp residue in helix three as the binding site for the ammonium nitrogen of agonists and antagonists. Strong evidence is found for a function as hydrogen-bonding sites of the serines in helix five for dopaminergic, adrenergic and serotonergic agonists.[44–47] Although the structure of melatonin is quite different from the neurotransmitters studied, which all have a charged amine, it is also a GPC receptor. Evolutionary it is reasonable to assume that the G-protein of the melatonin receptor is also connected to the third helix and as a consequence one binding place should be present in this helix. Based on these indications, found in the whole family of GPC receptors, special attention was focused on these helices, where we searched for possible binding sites which could accommodate melatonergic agonists.

Table 1. Energy values (kcal/mol) and relative affinities of melatonin receptor complexes and ligands

Compound	Ligand s^a	Complex[b]	Ligand[c]	Ligand m^d	Rel K_i[e]
Melatonin 1	18.96	−479.91	19.86	18.96	1.00
2	6.59	−492.65	7.40	6.59	0.003
3	9.36	−485.60	10.58	8.63	44
4	22.19	−481.59	23.09	22.19	1.6
5	20.65	−483.48	21.49	20.09	370
6	10.26	−483.71	10.33	9.85	1350

[a]Minimized energies (rms = 0.01) of the starting conformations.
[b]Minimized energies (rms = 0.1) of the complexes.
[c]Energy of the extracted ligands from the complex.
[d]Minimized energies (rms = 0.01) of the ligands.
[e]K_i-values of compounds **2–6** relative to melatonin (= 1).
The K_i values were determined in different experiments with variable K_D values for melatonin.
Affinities of melatonin for the ML-1 receptor in chicken brain determined with iodomelatonin as ligand.
K_D melatonin 0.57 nM for displacement of compound **2**.
K_D melatonin 0.39 nM for displacement of compounds **3** and **6**.
K_D melatonin 0.59 nM for displacement of compounds **4** and **5**.

Although melatonergic compounds are not yet that abundantly present, during the last years some series of different chemical structures were synthesized. From classical structure–activity relationship studies, it has been suggested that the amide-N**H**, the amide C**O**, and the methoxy-**O** are important functional moieties for affinity and agonistic activity.[48]

In an indirect modeling study, melatonin, the naftylamide and the more rigid compounds (as the amidotetralin, the amidoindan and the amidocarbazoles) were used to perform an extensive conformational search. From all the conformations found for the

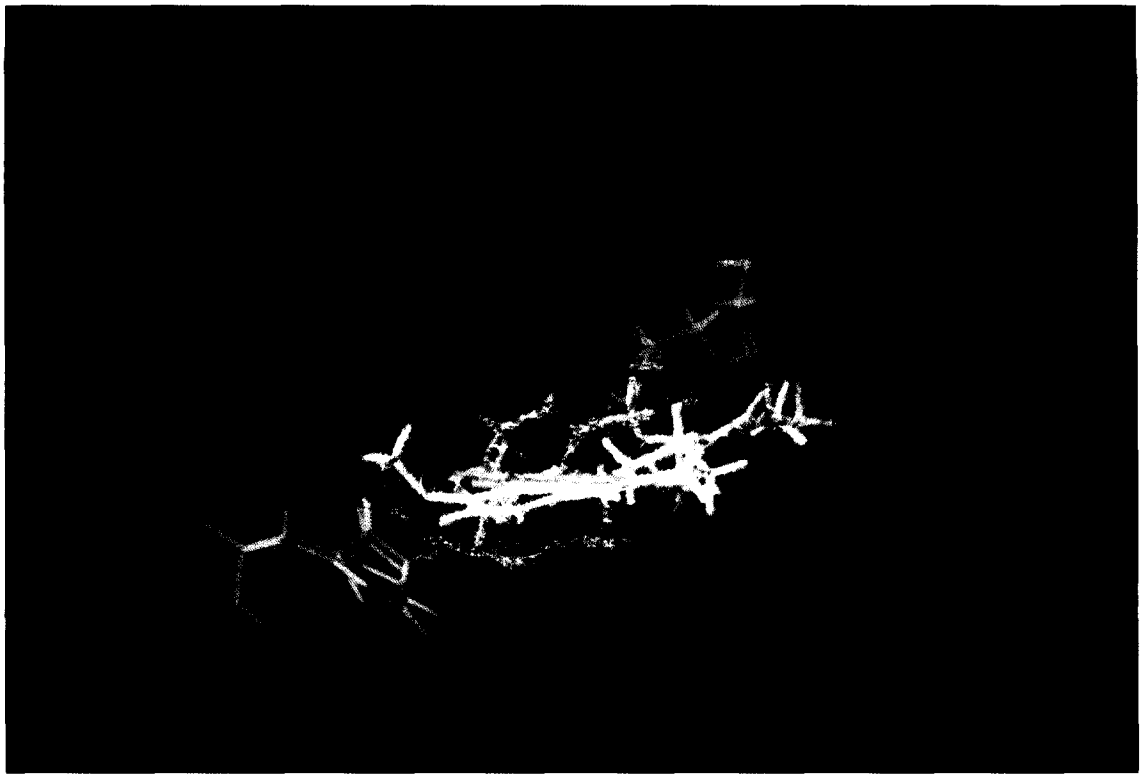

Figure 6. Volumes of melatonin agonists. The compounds **5** and **6** and their common volume (pink) togerher with the common volume of the compounds **1–4** (cyan) in the melatonin receptor.

different compounds, a cluster was constructed in which vectors of the functional groups in the these compounds matched the best.

For the interaction points of these functional groups at the receptor level, we suggest that they consist of the two serines in helix three and the histidine in helix five. The amide group of melatonin could interact with the serines 115 and 119, one being a hydrogen-bond donor and the other one a hydrogen-bond acceptor, while the histidine-H could form a hydrogen-bond with the methoxy-O (Fig. 4). In this case histidine is a good residue, for as is known, the methoxy-substituted compounds are melatonin agonists, while the hydroxy-substituted ones are not, indicating an interaction with a residue capable of being only a hydrogen-bond donor. Besides the directly acting serines and histidine possible stabilizing interactions[49] might take place with the aromatic rings of the phenylalanines 168 and 287 and the tryptophane 280. The residue Trp 256 functions as a steric boundary and will limit the total volume of direct-acting agonists.

In using the idea of mutual influence of ligand and receptor residues, the compounds were placed into the receptor. Special attention was paid to the mutual influence of ligand and receptor.

As could be expected from a mutual interaction of receptor and ligand, the serines and the histidine residues adopted different positions, depending upon the position of the compounds. For most compounds these variations in position were relatively small, except for the amidotetralin and the indan where Ser 115 deviated around 60° (Fig. 5).

In two compounds the methoxy group had to be moved out of the plane of the aromatic ring (**3** and **6**) to prevent steric interaction. After minimization of the complex of these compounds, a conformation was found, which led after minimization of the extracted ligand to a new local minimum. After minimization of the receptor complex with these compounds, a small conformational adaptation took place, which gave after minimization of the extracted ligand a new local minimum. A new minimum was also found for the carbazole **5**. The other compounds (**1**, **2** and **4**) ended up in their minimized starting conformations (Table 1). The relatively low affinities for compounds **5** and **6** might be due to deviations from their optimized starting conformations,[43] but also the conformational properties of the compounds within the receptor might prevent a proper orientation. This can be seen by a comparison of the common volume of the compounds **1–4** (the receptor excluded volume) with the volume of the less active compounds (**5** and **6**) where a region occupying additional space might be detrimental for an optimized binding (Fig. 6).

Conclusion

In this study we propose an interaction site in the melatonin receptor based on the common positions of a series of melatonin agonists, as derived from an indirect modeling study. This interaction site consists

of the serines in helix three and the histidine in helix five, forming hydrogen bonds with the ligands.

This study should be considered as a starting model to probe site-directed point mutations as well as a base for a study of the specific interaction energies between the ligands and the residues.

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