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The cannabinoid receptor: Computer-aided molecular modeling and docking of ligand

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A three-dimensional model of human cannabinoid receptor is constructed using computer-aided molecular modeling techniques. The helices of bacteriorhodopsin were used as the initial template to construct the transmembrane helices. The extracellular and intracellular loops were added using the SYBYL molecular modeling package. The extracellular N terminus was modeled on the basis of its similarity to rat oncomodulin. Similarly, the C terminus was constructed on the basis of similarity to bovine prothrombin fragment 1. The final structure was refined by several runs of minimization and dynamics calculation using the CHARMm package. Δ^9-Tetra hydrocannabinol was docked into the internal cavity using the AUTODOCK program. Our study shows that there may be a calcium-binding site in the extracellular N terminus of this receptor. The ligand binds mainly to a hydrophobic site, which consists of residues Met-240, Trp-241 (TMH-4), Trp-356, Leu-359, Leu-360 (TMH-6), and Ala-283 (TMH-5). Its phenolic hydroxyl group forms a hydrogen bond with the carboxy group of Ala-198 (TMH-3). The results of modeling agree well with experimental QSAR studies. © *1997 by Elsevier Science Inc.*

INTRODUCTION

The mechanism of action of cannabinoids such as Δ^9-tetrahydrocannabinol (d-THC) is of great interest to neuropharmacologists. The initial isolation of cDNA of a cannabinoid receptor from rat cerebral cortex cDNA library by Matsuda et al.[1] and subsequently in human brain[2] and peripheral tissue[3] has provided the basis for understanding the mechanism of action of this group of compounds at the molecular level. It has been shown that these receptors are members of the G protein-coupled receptor (GPCR) superfamily.[1-3] Members of this group of receptors share several structural features, such as the presence of seven transmembrane helical domains.[4] The structure of only one member of this group of receptors, namely bacteriorhodopsin, is available.[5] Several groups have constructed the three-dimensional structures of the helical part of other receptors on the basis of the structure of this protein.[6,7] It is believed that the cavity that is formed by the transmembrane helices is the ligand-binding site of these receptors. The present work was carried out to construct a three-dimensional model of the human cannabinoid receptor (HCNBR) and to dock Δ^9-tetrahydrocannabinol (d-THC) into the receptor-binding site to shed some light on the molecular basis of d-THC action.

METHODS

Construction of transmembrane helices

The initial position of the transmembrane helices in the sequence of human cannabinoid receptor (HCNBR) was taken as reported by Gerard et al.[2] The sequence of each helix plus four extra amino acids at each end was aligned with that of corresponding helix of bacteriorhodopsin, using the MULTALIGN program.[8] This procedure was carried out to give a better positioning of hydrophobic residues (which point toward the membrane phase) and hydrophilic residues (which point toward the ligand-binding cavity). Amino acid residues of bacteriorhodopsin were then mutated to those of the human cannabinoid receptor to construct the initial structure of the helices of this receptor. Intra- and extracellular loops of the human cannabinoid receptor were constructed using the LOOP option of the SYBYL molecular modeling package.[9]

Construction of the N-terminal region

The Protein Data Base was searched, using the FASTA package,[10] for proteins with sequences similar to that of the first 120 residues of HCNBR. The residues of the most similar protein (rat oncomodulin[11]) were mutated to the corresponding resi-

Color Plates for this article are on page 179.

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dues of the N-terminal part of HCNBR (i.e., the side chains of the residues of rat oncomodulin were changed to that of HCNBSR). This model was added to the structure of the helices.

Construction of the C-terminal region

The Protein Data Base was searched, using the FASTA package,[10] for proteins with sequences similar to that of the last 76 residues of HCNBR. The residues of the most similar protein (prothrombin fragment 1[12] were mutated to the corresponding residues of the C-terminal part of HCNBR to construct a model of the HCNBR C terminus. This model was added to the rest of the modeled structure.

Optimization of the modeled structure

The crude structure was further refined by several runs of molecular minimization and dynamics calculation using the CHARMm force field and the QUANTA package.[13] All-Atoms parameters and a dielectric constant of unity were used in these calculations. A disulfide patch was applied to the disulfide bond between residues 382–386 and 415–431. To avoid drastic movement of transmembrane helices, a harmonic constraint of 100 was applied to the α-carbons (CAs) of the residues of the transmembrane helices (Table 1). Minimization was carried out for 5 000 runs before dynamics calculations. For dynamics calculations the molecule was heated to 400 K for 5 ps and the temperature was increased in steps of 25 K/0.1 ps.

Docking of the ligand

The structure of Δ^9-tetrahydrocannabinol (d-THC) was constructed with the aid of the SYBYL package[9] and further refined using MOPAC.[14] The AM1 set of parameters[15] was used both for geometry optimization and charge calculation. Docking of ligand into receptor was carried out using the AUTODOCK set of programs.[16] The final structure of HCNBR, which had been obtained after several runs of minimization and dynamics calculations, was used for docking. For the initial position, d-THC was placed inside the transmembrane helix cavity roughly at the place corresponding to that of retinol in bacteriorhodopsin. The AUTODOCK program searches for the best conformation and best place of binding of the ligand within a fixed protein structure. Several hundred runs of space search were carried out to find the best ligand–receptor binding arrangement.

RESULTS AND DISCUSSION

The modeling of the human cannabinoid receptor was carried out in four steps: (1) determination of the transmembrane

Table 1. Alignment of transmembrane helices of human cannmabinoid receptor with that of bacteriorhodopsin[a]

Protein	Sequence
TMH-1	PEWIWLALGTALMGLGTLYFLVKG
1brd	* * * * *
HCNBR	PSQQLAIAVLSLTLGTFTVLENLLVLCVILHSDSLR
TMH-2	KFYAITTLVPAIAFTMYLSMLL
1brd	* :
HCNBR	PSYHFIGSLAVADLLGSVIFVYSFIDF
TMH-3	PIYWARYADWLFTTPLLLLLDLALL
1brd	: ** * *
HCNBR	DSRNVFLFKLGGVTASFTASVGSLFLTAIDRYI
TMH-4	TILAIVGADGIMIGTGLVGAL
1brd	: * * * *
HCNBR	TRPKAVVAFCLMWTIAIVIAVLPLLGWNCEKL
TMH-5	WWAISTAAMLTILYVLFFGF
1brd	* : :*:* * :
HCNBR	PHIDETYLMFWIGVTSVLLLFIVYAYMYILWKAHS
TMH-6	EVASTFKVLRNVTVVLWSAYPVVWL
1brd	* * * : : * :
HCNBR	DIRLAKTLVLILVVLIICWGPLLAIMVYDVFG
TMH-7	NIETLLFMVLDVSAKVGFGLIL
1brd	:* : : : *
HCNBR	NKLIKTVFAFCSMLCLLNSTVNPIIYALRSKDL

[a] HCNBR, Human cannabinoid receptor; 1brd, bacteriorhodopsin.

helices, (2) construction of loops connecting these helices, (3) construction of the N and C termini, and (4) minimization of the whole structure.

The initial locations of residues that make up the transmembrane helices were taken as reported by Gerard et al.[2] To determine their positions more accurately, each helix was compared with the corresponding helix of bacteriorhodopsin. The alignment of helices of human cannabinoid receptor with that of bacteriorhodopsin is shown in Table 1. Each transmembrane helix (TMH) of HCNBR is located as follows:

TMH-1: Val-121 to Ser-144

TMH-2: His-154 to Ile-175

TMH-3: Phe-189 to Ile-212

TMH-4: Ala-233 to Leu-253

TMH-5: Trp-279 to Leu-298

TMH-6: Arg-340 to Val-365

TMH-7: Ala-380 to Ser-401

These residues are similar to the helical residues that have been reported by Bramblett et al.[17] They used periodicity calculation to determine the location of helices in the HCNBR sequence. Comparing their results with the positions of the helices of our model of HCNBR (see above) showed that the positions of the first, third, sixth, and seventh helices are identical. However, their second and fifth helices are one turn longer at their C-terminal ends and their fourth helix is one turn longer at both ends. This would be expected because the fourth helix of bacteriorhodopsin is much shorter than those of other GPCRs. It has been suggested that the orientation of helices of GPCRs may not be similar to that of bacteriorhodopsin. To overcome this problem, instead of constructing only the helices, we have modeled the whole structure and minimization and dynamics calculations were carried out on the whole structure to allow the helices to take their position with respect to all constraints that may be exerted on them by the rest of molecule. The final structure after several runs of minimization and dynamics calculations is shown in Color Plates 1 and 2. As can be seen from Color Plate 2, the positions of helices are moved with respect to those of bacteriorhodopsin. Therefore, it is necessary to model the whole structure of any GPCR to determine the correct positioning of the helices and ligand-binding cavity.

It has been found that the N terminus of HCNBR is quite similar to that of rat oncomodulin[11] (Table 2). Rat oncomodulin is a calcium-binding protein and has two calcium-binding sites. One of these binding site (residues 92–102), which corresponds to residues 71–81 of HCNBR (Table 2), is conserved in HCNBR and may constitute a calcium-binding site. This calcium-binding site is shown in Color Plate 3. The effect of calcium concentration on the activity of the cannabinoid receptor has not been reported. However, it is known that other cations, e.g., sodium, can affect the ligand binding of the opiate receptor, another GPCR.[18] It would be interesting to study the effect of calcium on the ligand-binding activity of HCNBR.

The C-terminus part of HCNBR is similar to a prothrombin fragment 1[12] (Table 3). It is believed that this part of prothrombin binds to the membrane.[12] Considering the location of C-terminus of GPCRs, it is probable that the C terminus of HCNBR could also bind to the membrane.

As has been reported by Bramblett et al.,[17] the usual extracellular disulfide bond connecting extracellular loops is missing in the human cannabinoid receptor. However, there are possibly two other disulfide bonds. The first one is between Cys-382 and Cys-386 in TMH-7. This bond may contribute to the stability of the seventh transmembrane helix. The second disulfide bond is between Cys-415 and Cys-431 in the C-terminal part of the molecule. A similar disulfide bond also exists in the structure of prothrombin fragment 1,[12] which has been used as a template to model the C-terminal part of HCNBR.

The aim of any molecular modeling project is to provide

Table 2. Alignment and similarity between the N-terminus part of human cannabinoid receptor and rat oncomodulin[a]

Protein	Sequence
	10 20 30
HCNBR	MKSILDGLADTTFRTITTDLLYVGSNDIGYEDIKGD
	||:: : : ::: : :::::: || ::||
lomd	DILSAEDIAAALQECQDPDTFEPQKFFQTSGLSKMSASQVKDIFRFIDNDQSGY--LDGD
	10 20 30 40 50 60
	40 50 60 70 80 90
HCNBR	MASKLGYFPQKFPLTSFRGSDFQEKMTAGDNPQLVPADQVNITEFYNKSLSSFKENEENI
	:| || |||: :: |: : |::: |::: ::::: :||
lomd	---ELKYFLQKFQSDA-RELTESETKSLMDAADNDGDGKIGADEFQEMVHS
	70 80 90 100
	100 110 120
HCNBR	QCGENFMDIECFMVLNPSQQLAIA

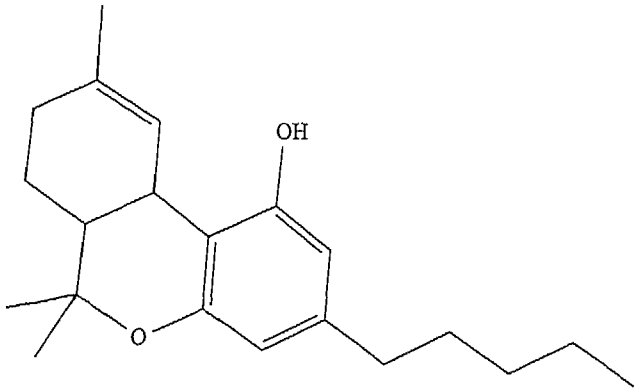
[a] HCNBR, Human cannabinoid receptor; lomd, rat oncomodulin.

Table 3. Alignment and similarity between the C-terminus part of human cannabinoid receptor and prothrombin fragment 1, residues 1–156[a]

Protein	Sequence
	400 410 420
HCNBR	YALRSKDLRHAFRSMFPSCEGTAQPLDNSMG
	||:| : :||:::::::||:: :| ::
2pf2	ANKGFLXXVRKGNLXRXCLXXPCSRXXAFXALXSLSATDAFWAKYTACESARNPREKL--
	10 20 30 40 50
	430 440 450 460 470
HCNBR	DSDCLHKHANNAASVH-RAAESCIKSTVKIAKVTMSVSTDTSAEAL
	:::||:: : ::::::: |:: | ::|:::
2pf2	-NECLEGNCAEGVGMNYRGNVSVTRSGIECQLWRSRYPHKPEINSTTHPGADLRENFCRN
	60 70 80 90 100 110

[a] HCNBR, Human cannabinoid receptor; 2pf2, prothrombin fragment 1, residues 1–156.

some information regarding the molecular basis of ligand–receptor interaction. To provide this, docking of d-THC with the modeled receptor was carried out using the AUTODOCK program.[16] This program uses simulated annealing techniques to search protein space and ligand conformation for the lowest energy complex. After several hundred runs of simulation, the complex with lowest energy was determined and is shown in Color Plate 4. As can be seen, d-THC could be accommodated in the internal cavity of the transmembrane helices. It should be mentioned that the idea of a common ligand-binding area within the transmembrane helices has been challenged.[19] However, considering the similar hydrophobic nature of d-THC (Figure 1) and retinol, it is justified to initially place the d-THC in the retinol position within transmembrane helices. The side chain and rings of d-THC bind to a hydrophobic pocket, which is made up from the aromatic rings of Trp-356 (TMH-6) and Trp-241 (TMH-4), and the side chains of Met-240 (TMH-4), Leu-359, Leu-360 (TMH-6), and Ala-283 (TMH-5) of human cannabinoid receptor. It should be mentioned that Trp-241 is highly conserved among GPCRs.[17] The phenolic hydroxy group of d-THC forms a hydrogen bond with the carboxy group of Ala-198 (TMH-3) (Color Plate 4). Met-383 (TMH-7) and Ala-244, Ile-247, and Ala-248 (TMH-4) also participate in the binding. It is believed that TMH-6 is involved in the coupling of the receptor with G protein.[4] The results of docking agree with three-dimensional quantitative structure–activity relationship (QSAR) studies,[20] which showed that steric repulsion behind the C ring is associated with decreased activity, whereas the steric bulk of the side chain contributes to increased binding affinity. The structure–activity relationship studies have shown that (1) the conformation of the C ring at the C-9 position, (2) the A ring phenolic hydroxy group, and (3) the hydrophobic side chain are important determinants for the production of analgesia, as well as other cannabinoid effects.[20]



Δ^9-Tetrahydrocannabinol

Figure 1. The structure of Δ^9-tetrahydrocannabinol, the main ligand for human cannabinoid receptor.

In conclusion, the modeled structure of human cannabinoid receptor is energy stable and whilst inevitably tentative agrees well with experimental data regarding ligand–receptor interactions and therefore could be used in such studies.

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The coordinate of the receptor is available from author on request (e-mail address: massoud@nrcgeb.ac.ir).

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