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Molecular modeling of μ opioid receptor and receptor-ligand interaction[1]

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KEY WORDS mu opioid receptors; molecular models; ligands; binding sites; fentanyl; structure-activity relationship

AIM: To construct the 3D structural model of μ opioid receptor (μOR) and study the interaction between μOR and fentanyl derivatives. **METHODS**: The 3D structure of μOR was modeled using the bacteriorhodopsin (bRh) as a template, in which the alignments of transmembrane (TM) of bRh and μOR were achieved by scoring the alignment between the amino acid sequence of μOR and the structure of bRh. The fentanyl derivatives were docked into the 7 helices of μOR and the binding energies were calculated. **RESULTS**: (1) The receptor-ligand interaction models were obtained for fentanyl derivatives. (2) In these models, the fundamental binding sites were possibly Asp147 and His297. The negatively charged oxygen of Asp147 and the positively charged ammonium group of ligand formed the potent electrostatic and hydrogen-binding interactions. Whereas the interactions between the positively charged nitrogen of His297 and the carbonyl oxygen of ligand were weak. In addition, there were some $\pi - \pi$ interactions between the receptor and the ligand. (3) The binding energies of the receptor-ligand complexes had a good correlation with the analgesic activities ($-\lg ED_{50}$) of the fentanyl derivatives. **CONCLUSION**: This model is helpful for understanding the receptor-ligand interaction and for designing novel μOR selective ligands.

Fentanyl was a potent analgesic[1]. Its prominent bioactivity and characteristic structure attracted a great attention. Four enantiomers of 3-methylfentanyl (Met) and 8 enantiomers of ohmefentanyl (Ohm) were synthesized in our laboratory[2,3]. There were tremendous stereo-differences between their analgesic activities and affinities for μ opioid receptor (μOR). Like many other fentanyl derivatives, they were selective μOR agonists[4].

The amino acid sequence of μOR from rat and human was determined from its cDNA sequence[5,6]. The rat μOR consisted of 398 amino acid residues and the N-terminal was in the extracellular space, the C-terminal in the cytoplasm.

In this paper, the three-dimensional (3D) structural model of μOR was constructed using the structure of bacteriorhodopsin (bRh) as a template. Then, the receptor-ligand interaction of fentanyl analogs was investigated by studying the bioactive conformations of ligands in the receptor and the relationship between the binding energies and the bioactivities of the ligands.

METHODS

Primary sequence comparison between μOR and bRh as well as hydropathicity analysis were made to define the putative transmembrane (TM) regions. The 7 TM domains were transformed into α-helices with the normal φ and Ψ values of $-58°$ and $-47°$, respectively, except for proline residue with $\varphi - 75°$, which led to slightly bent α-helices. The refined model of bRh, which was obtained from the Protein Data Bank (entry 1BRD)[7], was used as the template for positioning the 7 α-helices of μOR.

Modeling was achieved with the molecular modeling package SYBYL 6.2[8]. The interactive modeling and display were performed on SGI XZ 4000 workstation. The receptor was optimized in SYBYL using molecular mechanics calculation with the following parameters: a distance-dependent dielectric constant of 5.0, nonbonded cut-off 0.8 nm, AMBER force field with Kollman all-atom charges, conjugate gradient minimization until the RMS energy gradient < 0.7 kcal·(mol·nm)$^{-1}$. First, the all sidechains of 7 helices were minimized to convergence, in which the

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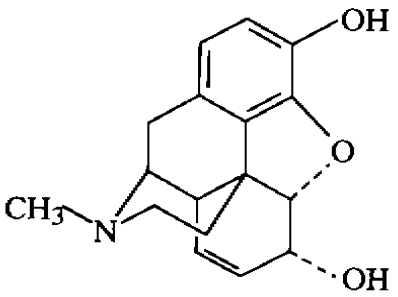
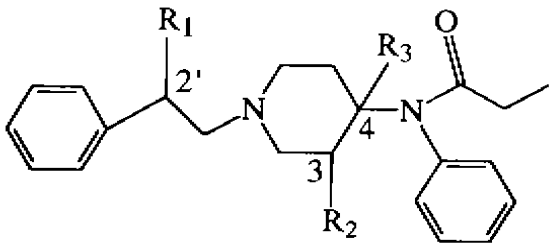
backbone of μOR was aggregated; then, the whole receptor was minimized to convergence.

The selective ligands for μOR were manually docked into their putative binding sites in the 7 helix bundles. To get the best possible interaction complexes, the docking procedure was repeated several times, simultaneously regulating different initial orientations for both the receptor side chains and the ligand. The complexes were optimized by molecular mechanics calculations using Tripos force field with Kollman all-atom charges for the receptor and Gasteiger-Huckel charges for the ligands. In this study, the 19 selective μOR ligands (shown below) were docked into the receptor model.



Morphine

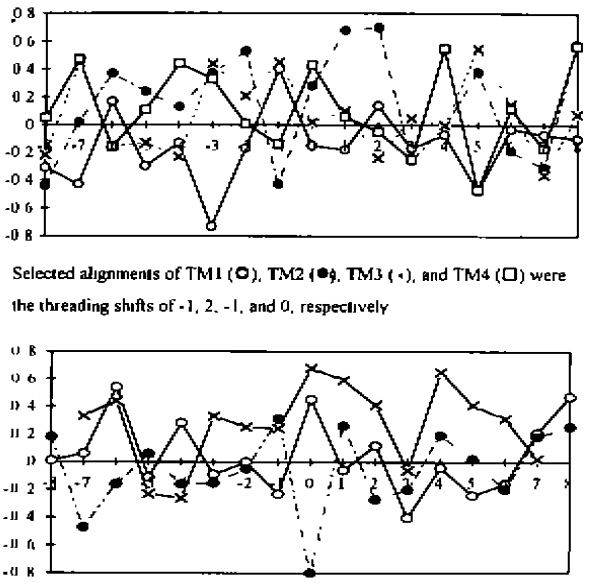


	R_1	R_2	R_3
Fentanyl	H	H	H
3-Methylfentanyl	H	CH_3	H (4 isomers)
Ohmefentanyl	OH	CH_3	H (8 isomers)
Carfentanil	H	H	$COOCH_3$
Lofentanil	H	CH_3	$COOCH_3$ (4 isomers)

RESULTS AND DISCUSSION

Modeling of μOR In 1990, a high quality 3D model for bRh was determined based on cryomicroscopy experiments[7]. The bRh receptor lacked sequence homology with G protein-couple receptor (GPCR), but it paralleled with GPCR in overall 3D structure patterns. The bRh had the similar functional characteristics and the structural patterns to rhodopsin[12], which had sequence homology with GPCR. It seems very likely that bRh and rhodopsin as well as other GPCR belong to the same structural class. It was reasonable that the 3D structure of μOR was modeled using the bRh structure as a template.

According to the method of scoring sequence-structure alignments[9], the plots of scores as a function of threading shift were obtained when threading the sequence of μOR through the bRh structure (Fig 1).



Selected alignments of TM1 (○), TM2 (●), TM3 (·), and TM4 (□) were the threading shifts of -1, 2, -1, and 0, respectively

Fig 1. Scoring alignments of μOR sequence and bRh structure.

Theoretically, the alignment with the highest score should be selected. On account of the complication of sequence alignment, the several alignments with higher score were tried to model μOR. The sequence alignments of the TM of bRh and μOR were chosen (Fig 2).

After energy minimization, the 3D model of μOR was achieved. In this model, the face directed toward the lipid bilayer mainly consisted of the nonaromatic hydrophobic residues, whereas the inside of the 7 helix bundles mainly of the conserved, polar residues. This arrangement was in accordance with the helical conformation of GPCR[10].

Due to the conformational flexibility of extra- and intra-cellular loop regions, the loop regions connecting individual helices were omitted in the present modeling. However, omission of the loop regions was not influential in analyzing the receptor-ligand interaction, because the ligand-binding pocket was supposed to be located in the region surrounded by transmembrane helices[7,10].

TM1	bRh	11	IWLALGTALMGLGTLYFLV KGM	32
	μOR	71	IMALYS I VCVVGLFGNFLVMYV	92
TM2	bRh	38	DAKKFYA I TTLVPAIAFTMYL SML	61
	μOR	107	I F NLALADALATS TLPFQSVNYLM	130
TM3	bRh	81	ARYADWLF T TPLLLLDL ALL	100
	μOR	142	IV I S I DYYNMFTSI FT LCTM	161
TM4	bRh	108	ILA LVGADGI MI GTGLVGAL	127
	μOR	186	IVNVCNWI LS SA IG LPVMFM	205
TM5	bRh	137	WWAI STAAMLYILYVLFFGFT	157
	μOR	234	I C VF IFA F I MPIL I ITVCYGL	254
TM6	bRh	167	VAS TFKVLR NVT VVLWSAYPV	187
	μOR	282	VLVVVAVF I VCWTP I H I YVI I	302
TM7	bRh	204	ETLLFMVLDVSAKVG FGLI	222
	μOR	321	CIALGY TNSCLNPV LYAFL	339

Fig 2. Sequence alignment of transmembrane regions of *bRh* and *μOR*.

Modeling of ligand-receptor complexes The 13 protonated ligands (including fentanyl, 4 enantiomers of Met, and 8 enantiomers of Ohm) were docked into the inside of 7 helix bundles. In docking procedure, the bioactive conformations of the ligands were chosen as our previous results[11].

We found 3 charged residues in TM region, ie, 2 negatively charged residues (Asp114 and Asp147) and 1 positively charged residue (His297). Of these amino acid residues, 2 Asp residues were conserved with all GPCR[13]. The site-directed mutagenesis showed that Asp147 residue played an important role in binding with agonist.

Our previous study[11] showed that, the distance between RP_1 and RP_2 was about 0.93 nm, corresponding to the distance between the 2 hypothetical site points binding with the protonated nitrogen of piperidine and the carbonyl oxygen of 4-phenylpropanamide of ligand, respectively. It was in accordance with the distance between the negatively charged oxygen of Asp147 and the positively charged nitrogen of His297 in our μOR model (Tab 1).

Therefore, the residues Asp147 and His297 would be chosen as the binding sites, which were consistent with the results of modeling receptor-lofentanil interaction[14].

After manual adjustment, the docking procedure was carried out to convergence. For each ligand-receptor complex, the geometric optimization was also performed to convergence. The 3D models of receptor-ligand complexes were obtained, one of

Tab 1. Geometric parameters of binding sites of μOR model.

Compounds	d_1[a]	d_1[b]	d_2[a]	d_2[b]	d_3[a]	d_3[b]	d_4[a]	d_4[b]
Fentanyl	0.569	0.582	0.797	0.923	0.695	0.718	1.060	1.026
(3R,4R)-Met	0.583	0.598	0.825	0.975	0.735	0.717	1.073	1.043
(3R,4S)-Met	0.583	0.583	0.803	0.937	0.716	0.715	1.089	1.078
(3S,4R)-Met	0.577	0.585	0.809	0.877	0.717	0.715	1.073	1.067
(3S,4R)-Met	0.521	0.585	0.799	0.963	0.706	0.717	1.040	1.014
(3R,4R,2′R)-Ohm	0.610	0.695	0.834	0.940	0.736	0.717	0.070	1.066
(3R,4R,2′R)-Ohm	0.610	0.694	0.822	0.918	0.727	0.715	1.086	1.108
(3R,4R,2′R)-Ohm	0.576	0.568	0.827	0.874	0.723	0.715	1.071	1.046
(3R,4R,2′R)-Ohm	0.582	0.578	0.825	0.970	0.709	0.718	1.043	1.022
(3R,4R,2′R)-Ohm	0.592	0.568	0.829	0.958	0.737	0.718	1.059	1.009
(3R,4R,2′R)-Ohm	0.611	0.666	0.820	0.803	0.725	0.715	1.081	1.074
(3R,4R,2′R)-Ohm	0.593	0.590	0.808	0.882	0.719	0.715	1.074	1.070
(3R,4R,2′R)-Ohm	0.558	0.578	0.786	0.958	0.707	0.718	1.054	1.006

[a] Calculated in this study; [b] obtained from Ref 11; d_1 (nm) is the distance between the phenyl center of phenylethyl of ligand and the positively charged oxygen of Asp147, d_2 (nm) the distance between the positively charged oxygen of Asp147 and the negatively charged nitrogen of His297, d_3 (nm) the distance between the negatively charged nitrogen of His297 and the phenyl center of 4-phenylpropanamide of ligand, d_4 (nm) the distance between two phenyl rings of ligand.

which was shown in Fig 3.

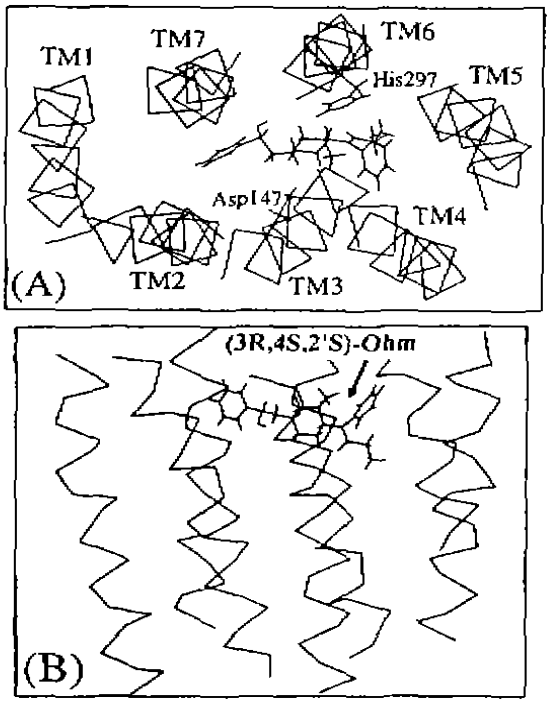


Fig 3. Stereoplots of the (3R,4S,2'S)-Ohm in the binding sites. Only 2 residues (Asp147 and His297) and the trace of α-carbon atoms of μOR. (A) Top view of the cell; (B) Side view of the membrane.

According to the above models, the possible binding sites were mainly defined by the following amino acids: Asp147 (TM3), His297 (TM6), Tyr148 (TM3), Trp192 (TM4), and Tyr326 (TM7), which were corresponding to the 4 key moieties of μ pharmacophore (Fig 4).

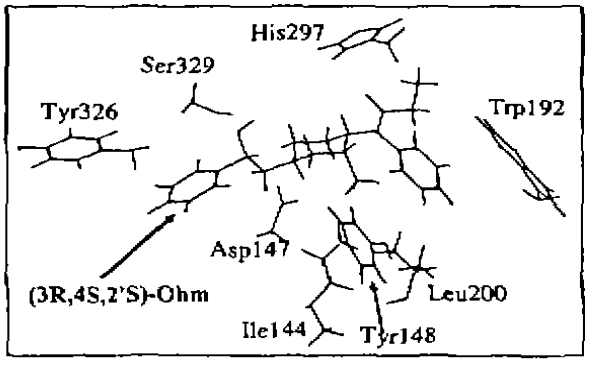


Fig 4. Four key moieties of μ pharmacophore of fentanyl derivatives interacting with putative binding sites of μOR, shown for (3R,4S,2'S)-Ohm.

There were a number of interactions between the ligands and the amino acid residues in the receptor-ligand complex model. The potent electrostatic and hydrogen-binding interactions occurred from the negatively charged oxygen of Asp147 of the receptor to the positively charged ammonium group on piperidine ring of ligand, whereas the weak electrostatic and hydrogen-binding interactions between the positively charged nitrogen of His297 and the carbonyl oxygen of 4-phenylpropanamide of ligand. There took place some π − π interactions of receptor with ligands; the 2 aryl rings of residues Tyr148 and Trp192 interacted with the phenyl ring in 4-phenylpropanamide of ligand, which was inserted between 2 above aryl rings; whereas the aryl ring of Tyr326 with the phenyl ring in *N*-phenylethyl of ligand.

Binding energies of the receptor-ligand complexes The binding energy ($E_{binding}$) of each ligand with μOR was calculated as the follows:

$$E_{binding} = E_{complex} - E_{ligand} - E_{receptor} \quad (1)$$

where E_{ligand} is the conformational total energy of the ligand corresponding to the bioactive conformation, and $E_{receptor}$ is the energy of the optimized receptor (Tab 2).

With Partial Least Squares (PLS) method, the regression equation between $-\lg ED_{50}$ and $E_{binding}$ was achieved:

$$-\lg ED_{50} = -3.101 - 0.080 \cdot (E_{binding}) \quad (2)$$

($r^2 = 0.761$, $F = 35.06$, $s = 0.617$)

Obviously, the binding energies of receptor-ligand complexes had a good correlation to the analgesic activties of ligands. The more negative the value of $E_{binding}$, the more potent the bioactivity of ligand.

However, the interaction energies obtained could not be used to calculate exact affinities of ligand-receptor, because changes in entropy and solvation were not taken into account.

Elucidation of structure-activity relationship (SAR) of ligands The receptor-ligand complex model could be used to explain the known SAR for the fentanyl analogs as below[15].

(1) Introducing a methyl into the 3-position of piperidine ring to form the 3R,4S-configuration of Met and Ohm enhanced their analgesic activities and

Tab 2. Binding energies of receptor-ligand interaction (kcal·mol^{-1}) and $-\lg ED_{50}$ (mol·kg^{-1}) in hot plate test on mice.

Compounds	$E_{complex}$	$E_{receptor}$	E_{ligand}	$E_{binding}$	$-\lg ED_{50}$
Fentanyl	−598.784	−491.908	19.902	−126.778	6.78
(3R,4R)-Met	−591.113		15.900	−115.105	6.42
(3R,4S)-Met	−585.272		41.215	−134.579	7.76
(3S,4R)-Met	−579.604		24.584	−112.280	5.69
(3S,4S)-Met	−594.605		19.060	−121.757	7.01
(3R,4R,2′R)-Ohm	−591.524		17.005	−116.671	6.71
(3R,4S,2′R)-Ohm	−577.826		45.154	−131.072	7.90
(3S,4R,2′R)-Ohm	−574.625		23.365	−106.082	4.57
(3S,4S,2′R)-Ohm	−591.333		18.059	−117.484	6.69
(3R,4R,2′S)-Ohm	−598.959		23.175	−130.226	7.58
(3R,4S,2′S)-Ohm	−591.252		57.501	−156.845	8.54
(3S,4R,2′S)-Ohm	−578.490		21.637	−108.219	4.57
(3S,4S,2′S)-Ohm	−591.709		29.012	−128.813	7.41
Morphine[a]	−536.662		20.984	−65.738	
Carfentanil[a]	−642.647		9.444	−160.183	
(3R,4R)-Lofentanil[a]	−620.602		10.852	−139.546	
(3R,4S)-Lofentanil[a]	−630.989		14.176	−153.257	
(3S,4R)-Lofentanil[a]	−583.584		10.786	−102.462	
(3S,4S)-Lofentanil[a]	−607.110		12.737	−127.939	

[a] Not including in the regression equation (2); due to the absence of comparable $-\lg ED_{50}$ data.

affinities for μOR.

From our model, the residues Ile144, Tyr148, and Leu200 could form one small hydrophobic pocket, which might be occupied only by one methyl group and resulted in increasing hydrophobic interaction with ligands. So, the 3R, 4S-configuration of Met and Ohm had extremely higher analgesic activities. Changing this methyl group into allyl or propyl, the activities distinctly decreased, because the group was too long to enter the small hydrophobic pocket.

Contrarily, converting the above configuration into the 3S,4R-configuration of Met and Ohm, the activities dramatically decreased, because the methyl group in this configuration escaped from the small hydrophobic pocket and had the steric hindrance with Tyr148.

(2) Introducing a hydroxyl group into the 2′-position of phenylethyl increased the analgesic activity of ligands, which could be explained by forming electrostatic and hydrogen-binding interactions between Ser329 and this 2′-hydroxyl group. This interaction is very obvious in receptor-(3R,4S,2′S)-Ohm complex (Fig 4).

(3) To lengthen or shorten the chain of phenylethyl substituent led to a sharp decrease in analgesic activity. This trend was because the cavity within the 7 helix bundles of μOR model could not accommodate too long ligands; otherwise, small ligands could not efficiently interact with the binding sites.

(4) Replacement of the phenyl group in phenylethyl by some other rings, eg, aromatic heterocyclic, planar cyclic and planar-like oxygen-containing cyclic rings, might ensure certain analgesic activity. But the phenyl group was still the best, because other ring structures weakened the $\pi-\pi$ interaction with Tyr326.

(5) To introduce a nucleophilic or eletrophilic group into the phenyl of 4-phenylpropanamide generally resulted in a sharp decrease of analgesic activity. This was due to a weakening of the $\pi-\pi$ interactions with Tyr148 and Trp192 and producing seriously steric hindrance with nearby amino acid residues.

To conclude, the μOR model achieved in this study is meaningful for understanding the receptor-ligand interaction and probably helpful for designing novel μOR selective ligands.

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μ 阿片受体及受体-配基相互作用的分子模拟

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关键词 μ 阿片受体；分子模型；配基；结合位点；芬太尼；结构-活性关系

目的：构建 μ 阿片受体（μOR）的三维结构模型并研究它与芬太尼衍生物的相互作用。方法：以细菌视紫红质为模板，模拟 μOR 的三维结构；然后，将芬太尼衍生物对接到 μOR 的七个 α 螺旋束之内，并计算结合能。结果：(1)得到受体－配基作用模型。(2)模型中，基本结合位点可能是 Asp147 和 His297。Asp147 与配基的正电性铵基形成强的静电和氢键相互作用，这种作用在 His297 和配基的羰基 O 原子之间较弱。受体、配基间还存在某些 π－π 相互作用。(3)受体-配基结合能与芬太尼衍生物的镇痛活性间有良好的相关性。结论：模型有助于理解受体-配基的相互作用和设计新的阿片 μ 选择性配基。