BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Mar; 17 (2): 156 - 160

Molecular modeling of μ opioid receptor and its interaction with ohmefentanyl[1]

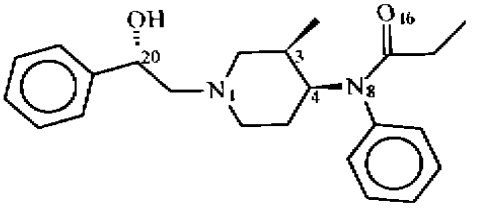
TANG Yun, CHEN Kai-Xian, JIANG Hua-Liang, WANG Zhi-Xian, JI Ru-Yun, CHI Zhi-Qiang
(*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China; Shanghai Research Center of Life Sciences, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS molecular models; mu opioid receptor; ohmefentanyl; analgesic; 3D structure-activity relationship

AIM: To build up the structure model of μ opioid receptor, then combined with the receptor model, to investigate the action mechanism of ohmefentanyl on the receptor. **METHODS**: Using the three-dimensional structure of bacteriorhodopsin as a template, we constructed μ opioid receptor model on computer. Ohmefentanyl was then docked into the supposed receptor binding sites. **RESULTS**: A good ligand-receptor interaction model was achieved. The possible binding sites were found to be Asp147 and His319. The protonated N atom of ohmefentanyl form potent electrostatic and hydrogen-bonding interactions with residue Asp147 of the receptor, the O atom of the carbonyl group form weak electrostatic and hydrogen-bonding interactions with residue His319, and the two phenyl groups form π-π interactions with some aryl residues of the receptor around ligand. **CONCLUSION**: The ligand-receptor interaction model should be helpful for rational design of novel analgesic.

Ohmefentanyl (Ohm), first synthesized in Prof CHI's Laboratory in the early 1970s, is the most potent and most selective agonist of μ opioid receptor[1]. Dr WANG Zhi-Xian synthesized its 8 enantiomers. Their analgesic activities varied extremely[2]. These provided powerful background for us to understand how Ohm to bind and act on the μ opioid receptor.

The primary sequences of rat and human μ opioid receptors have been recognized from gene cloning[3,4]. The rat μ opioid receptor consists of



(3R, 4S, 20S)-ohmefentanyl

398 amino acid residues and belongs to the superfamily of G-protein coupled receptors (GPCR), which is a single chain having 7 transmembrane (TM) segments with the NH_2 terminal in the extracellular space and COOH terminal in the cytoplasm. Using molecular modeling method, we predict the three-dimensional (3D) structural model of the receptor from its primary sequence.

This paper is to construct the μ opioid receptor model and investigate the action mechanism of Ohm on the receptor.

METHODS

This work was performed on Silicon Graphics IRIS Indigo XZ4000 workstation using SYBYL V6.1 software package[5]. The structure data of bacteriorhodopsin (bR)[6] were read from the Protein Data Bank (PDB)[7]. The amino acid sequence of rat μ opioid receptor was inputted manually[3]. The structure of Ohm was built up and optimized interactively using the SYBYL program.

Sequence alignments and comparisons between μ opioid receptor and other GPCR, as well as bR, were carried out to define the putative TM regions. The sequences of the TM domains were converted into α-helices with normal φ and Ψ values of $-58°$ and $-47°$ separately in SYBYL/Biopolymer module, except for proline residue with $\varphi = -75°$, which led to slightly bent α-helix. The structure of bR was used as the template for positioning of the α-helices main axes. Automatic adjustments were made to remove the unfavorable steric interactions. The geometry of the protein was optimized for 200 steps with the steepest descent minimizer and subsequently for 2000 steps with the conjugate gradient minimizer, using the Amber force field with Kollmann all-atom charges. A

[1] Project supported by the Chinese National "863" High Technology Program and the National Commission of Science and Technology of the People's Republic of China.
Received 1995-09-05 Accepted 1995-12-26

cutoff of 0.8 nm was used, while dielectric constant was set 5.0 and depended on the distance.

We generated all of the loop segments using Loop Search method in Biopolymer module, and formed an intramolecular disulfide bond between Cys140 and Cys217. The whole protein structure was also optimized by energy minimization.

Ohm was docked into the supposed binding sites among the 7 helix bundles. After manual adjustment, the whole complex was minimized using Tripos force field with Kollmann all-atom charges for the receptor and Gasteiger-Huckel charge for the ligand. The protonated Ohm and morphine were also docked into the receptor sites in the same way.

RESULTS

Modeling of the receptor According to the analysis of hydropathicity and referred to many other reports[8,9], the sequence alignment of the TM regions of bR and μ opioid receptor was shown in Fig 1.

```
TM1  bR    8 PEWIWLALGTALMGLGTLYFLVKGM  32
     μOR  70 TIMALYSIVCVVGLFGNFLVMYVIV  94

TM2  bR   38 DAKKFYAITTLVPAIAFTMYLSMLL  62
     μOR 105 IYIFNLALADALATSTLPFQSVNYL 129

TM3  bR   79 YWARYADWLFTTPLLLLDLALL    100
     μOR 140 CKIVISIDYYNMFTSIFTLCTM    161

TM4  bR  106 GTILALVGADGIMIGTGLVGA     126
     μOR 187 VNVCNWILSSAIGLPVMFMAT     207

TM5  bR  137 WWAISTAAMLYILYVLFFGFT     157
     μOR 234 ICVFIFAFIMPILIITVCYGL     254

TM6  bR  170 TFKVLRNVTVVLWSAYPVVWLI    191
     μOR 281 MVLVVVAVFIVCWTPIHIYVII    302

TM7  bR  202 NIETLLFMVLDVSAKVGFGLILLRS 226
     μOR 312 TFQTVSWHFCIALGYTNSCLNPVLY 336
```

Fig 1. Sequence alignment of the transmembrane regions of bacteriorhodopsin (bR) and μ opioid receptor (μOR).

After energy minimization, the whole receptor structure model was achieved. In this model, the hydrophobic residues lay mainly on the outer surface of the TM regions, contacting the lipid bilayer, whereas the polar residues lay mainly in loop regions and inner surface of the TM regions.

Modeling of the receptor-Ohm interaction (3R, 4S, 20S)-Ohm, the most potent Ohm conformer, and its protonated form were docked into the cavity near Asp147 and His319 with one of the oxygens of the carboxylate at Asp147 as an anchoring point, respectively. Because the structure of Ohm is very flexible, it is difficult to determine its active conformation. We carried out conformational searching to the 8 isomers by multi-search method. Each isomer got 100 conformers. Then a common conformation was attained by DISCO module. This common conformation was assumed as the bioactive one and used in the ligand-receptor interaction. After manual adjustment and geometric optimization, a good receptor-ligand interaction model was achieved (Tab 1, Fig 2).

Tab 1. Docking energy of ligand-receptor interaction (kcal·mol^{-1}).

Energy	Ohm	Protonated Ohm
Asp147 and His319		
Total	−142.075	−189.426
Steric	−131.683	−132.894
Electrostatic	−10.392	−56.532
Asp147 and His297		
Total	−114.724	−157.678
Steric	−102.959	−103.588
Electrostatic	−11.765	−54.090

From this primary model, the possible binding sites should be Asp147, Tyr148, Trp318, His319 (Fig 3).

The basic action moieties of fentanyl derivatives were two sites: one was N_1 atom on piperidinyl ring which formed potent electrostatic and hydrogen-bonding interactions with the carboxy group of residue Asp147 on TM3 of the receptor, the other was O_{16} atom in carbonyl group which pointed to the imidazolyl plane of residue His319 on TM7 and formed weak electrostatic and hydrogen-bonding interactions. One phenyl ring inserted into 2 aryl ring planes of residues Trp318 and His319 forming π-π interactions. The other phenyl ring formed π-π interaction with ring plane of Tyr148. All these electrostatic, hydrogen-bonding and π-π interactions render Ohm high binding affinity to μ opioid receptor, thus possessed potent analgesic activity.

DISCUSSION

Modeling of the receptor The fact that no GPCR have been crystallized and elucidated 3D

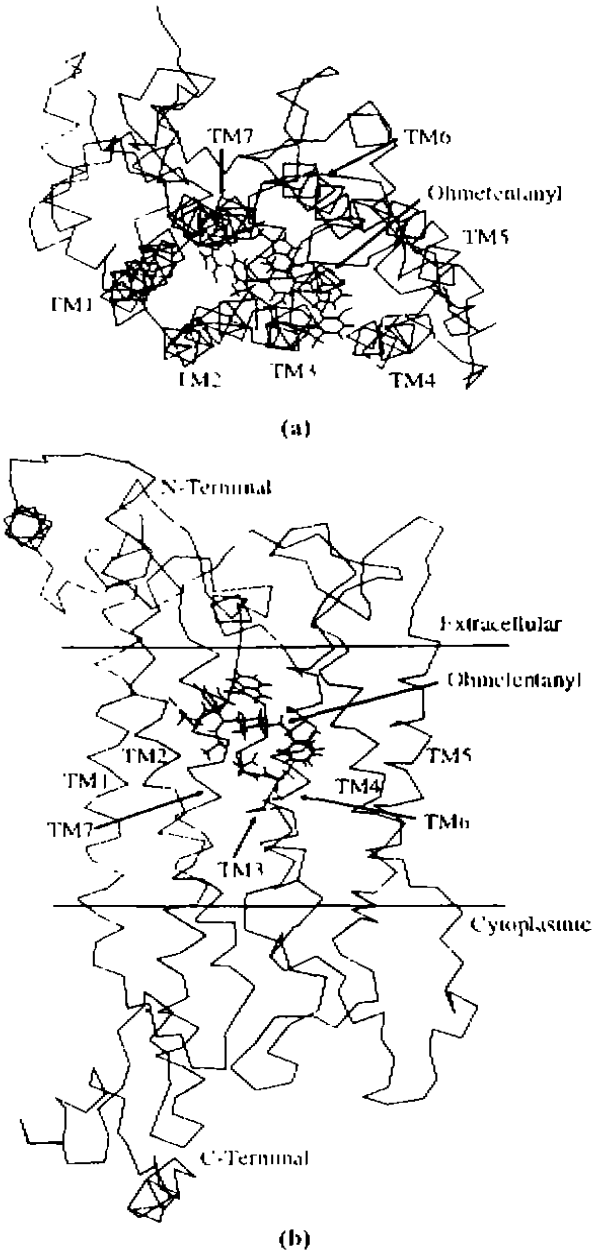


Fig 2. Display of the complex between (3R, 4S, 20S)-Ohm and the μ opioid receptor, shown in α-carbon atom only, except for residues Asp 147, Tyr 148, Trp 318 and His 319. (a) View of the complex from the outside of the cell; (b) side view of the ligand-receptor complex.

structures to date made it difficult for us to model the structure of μ opioid receptor. The only available structure is that of bR. Although bR does not belong to GPCR family and has low primary sequence homology with them, bR has the same ligand retinal and 7 TM α-helices as rhodopsin, a member of GPCR family, so we suggest that there

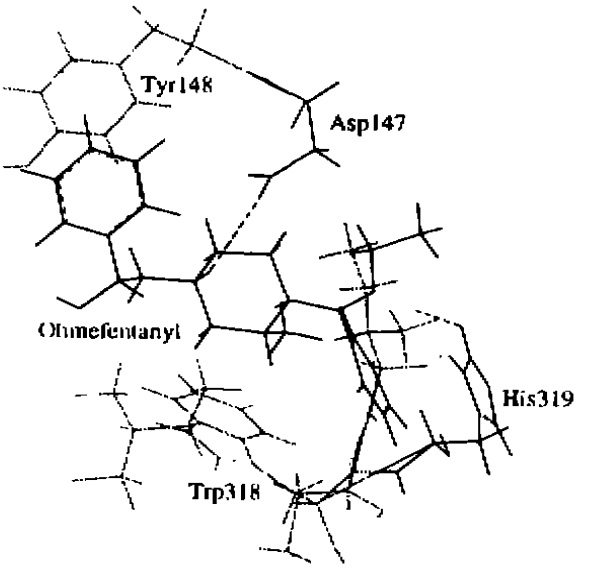


Fig 3. Protonated Ohm interaction with proposed binding sites of μ opioid receptor.

is high structure homology between bR and rhodopsin as well as other GPCR including μ opioid receptor. Recently a 0.9 nm resolution electron diffraction structure was obtained for bovine rhodopsin[10]. Its projection footprint indicated the presence of 7 TM α-helices in this receptor (and probably in all GPCR). Although the helix arrangement appeared to be different from that in bR, if we rotate 15° of the model around an axis perpendicular to the 7 helices, the 2 proteins would have the same projection images. Furthermore, different crystallization condition strongly affected the packing of the protein molecules[11]. Therefore, in the absence of 3D structure of μ opioid receptor, we built up its TM structure model using the structure of bR as the template.

Modeling of the loop regions is difficult, because loop regions are variable and no template could be used. However, we built the loops by searching the library of the PDB for protein fragments with Loop Search program, then selected the best conformation according to the fitting root mean square (RMS) and van der Waals contact values. Because of the least RMS and contact values, so the loop regions most closely matched the geometric requirement of bridging from one helix to the next. There are 2 highly conserved Cys residues in the 2nd and 3rd extracellular loops of the GPCR (Cys140 and

Cys217 in the μ opioid receptor). Site-directed mutagenesis results suggested that these residues played a key role in maintaining the active conformation of the receptor. Biochemical analysis of rhodopsin showed that the 2 Cys residues were involved in an intramolecular disulfide bridge and it was a structural feature common to the entire family of receptors[12]. Thus, we generated an intramolecular disulfide bond between Cys140 and Cys217.

This structural model remains to be confirmed by experiment, but it is helpful to understand the structure and function of the receptor and study the ligand-receptor interaction at present.

Determination of the binding sites The binding sites of μ opioid receptor have not been defined by experiment so far, that made it difficult to study the receptor-Ohm interaction. Some experimental evidences demonstrated that for peptide receptor, the nonpeptide ligand binding sites located in the 7 helix bundle[12], so it was supposed the Ohm-binding pocket being located in the TM regions. In the model of μ opioid receptor, there are 2 Asp residues in the TM regions: Asp114 and Asp147, 2 His residues: His297 and His319, and 1 Lys residue: Lys141. These 5 residues were all charged and the 2 Asp residues were conserved within most GPCR. One or more of those charged residues were responsible for the binding of the ligand.

In the previous paper, we had carried out quantum chemical calculation and QSAR analysis on 3-methylfentanyl derivatives[13]. The results showed that four portions of 3-methylfentanyl derivatives, ie charged atom N_1, charged atom O_{16}, 3-methyl, and phenyl of atom N_8, were important to their analgesic activities. At physiological pH values, these ligands would all display a high positive charge around the N_1 atom of the ligands due to protonation, whereas there is a negative center around atom O_{16}. So it was suggested that there must be corresponding negative and positive centers in the receptor. Residues Asp147 and His319 would be the first choice.

New site-directed mutagenesis experiment[14] has revealed that residue Asp147 took part in the binding with agonist directly, while deletion of 64 N-terminal and 33 C-terminal amino acids produced little effect on receptor function, which supported our model. His319 has not been studied by mutation because this residue would not be located in TM regions. However, in our model, His319 was not only included in TM regions, but also thought of as a critical residue. Alanine substitution for His297 did dramatically reduce agonist affinity while maintaining normal or increased intrinsic activity of the mutant receptor. Therefore, we also studied the complex of Ohm and μ opioid receptor with Asp147 and His297 as the negative and positive electric center, respectively (Tab 1). As shown in Tab 1, in former case, the ligand bound with the receptor more close than that in latter case. Therefore, we suggested residue His319 as the positive electric center instead of His297, and His297 should have relation with the recognition of ligand and receptor, but not participate in binding with the agonist directly. We docked morphine into the cavity between Asp147 and His319, a good interaction model was obtained, too.

Elucidation of the action mechanism In the receptor-ligand model, 3-methyl extended into a hydrophobic pocket formed residues Ile296, Trp318, Cys321, and Ile322, just matched each other. When Ohm was in 3S, 4R-configuration, steric hindrance occurred in 3-methyl and made the activity decreasing signficantly. The difference in position 20 was not significant to the activity, it cannot attribute to a strong hydrogen-bonding interaction. Molecular modeling showed that the hydroxyl group on position 20 pointed at the hydroxyl group of residue Tyr148 and formed weak hydrogen-bonding interaction, hydroxyl group in S-configuration forming hydrogen-bonding more easily than that in R-configuration.

The above mentioned interactive pattern could explain the different analgesic activities between fentanyl derivatives. Introducing a methyl group in the 3-position of the piperdine ring of fentanyl, namely 3-methylfentanyl, 3-methyl could increase the hydrophobic interaction, but it would be more important to affect the orientation and position of the phenyl ring on N_8 atom. That made the activity of 3-methylfentanyl more potent. Drawing into a hydroxyl in position 20 of 3-methylfentanyl, namely Ohm, could form a new hydrogen-bonding

156-160

interaction with the receptor, thus increase the activity.

In conclusion, we presented a preliminary μ opioid receptor-Ohm interaction model in this paper. With this model, we determined the possible binding sites of μ opioid receptor were Asp147, Tyr148, Trp318, and His319. This results were not only consistent with the mutagenesis data, but also explained the activity differences between fentanyl derivatives. In the next step, we will modify this receptor-ligand interaction model further by molecular dynamics and try to design novel analgesic.

REFERENCES

1 Xu H, Chen J, Chi ZQ. Ohmefentanyl-a new agonist for μ-opiate receptor. Sci Sin [Ser B] 1985; **28**: 504 - 11.

2 Wang ZX, Zhu YC, Chen XJ, Ji RY. Enantiomers of ohmefentanyl and its analgesic activity. Chin Sci Bull 1994; **39**: 2004 - 8.

3 Chen Y, Mestek A, Liu J, Hurley JA, Yu L. Molecular cloning and functional expression of a μ-opioid receptor from rat brain. Mol Pharmacol 1993; **44**: 8 - 12.

4 Wang JB, Johnson PS, Persico AM, Hawkins AL, Griffin CA, Uhl GR. Human μ opiate receptor cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. FEBS Lett 1994; **338**: 217 - 22.

5 SYBYL [computer program], version 6.1. St Louis (MO): TRIPOS Associates Inc, 1994.

6 Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckmann E, Downing KH. Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. J Mol Biol 1990; **213**: 899 - 929.

7 Berstein FC, Koetzle TF, Williams GJB, Meyer EF Jr, Brice MD, Rodgers JR, *et al*. The Protein Data Bank: a computer-based archival file for macromolecular structures. J Mol Biol 1977; **112**: 535 - 42.

8 Trumpp-Kallmeyer S, Hoflack J, Bruinvels A, Hibert M. Modeling of G-protein-coupled receptors: application to dopamine, adrenaline, serotonin, acetylcholine, and mammalian opsin receptors. J Med Chem 1992; **35**: 3448 - 62.

9 Donnelly D, Findlay JBC. Seven-helix receptors: structure and modelling. Curr Opin Struct Biol 1994; **4**: 582 - 9.

10 Schertler GFX, Villa C, Henderson R. Projection structure of rhodopsin. Nature 1993; **362**: 770 - 2.

11 Hoflack J, Trumpp-Kallmeyer S, Hibert MF. Re-evaluation of bacteriorhodopsin as a model for G protein-coupled receptors. Trends Pharmacol Sci 1994; **15**: 7 - 9.

12 Strader CD, Fong TM, Tota MR, Underwood D. Structure and function of G protein-coupled receptors. Annu Rev Biochem 1994; **63**: 101 - 32.

13 Tang Y, Wang HW, Chen KX, Ji RY. QSAR of 3-methylfentanyl derivatives studied with neural networks method. Acta Pharmacol Sin 1995; **16**: 26 - 32.

14 Surratt CK, Johnson PS, Moriwaki A, Seidleck BK, Blaschak CJ, Wang JB, *et al*. μ Opiate receptor: charged transmembrane domain amino acids are critical for agonist recognition and intrinsic activity. J Biol Chem 1994; **269**: 20548 - 53.

μ阿片受体及其与羟甲芬太尼相互作用的分子模拟

唐 赞，陈凯先，蒋华良，王智贤，稽汝运，池志强 （中国科学院上海药物研究所；中国科学院上海生命科学研究中心，上海 200031，中国）

关键词 分子模型；μ阿片受体；羟甲芬太尼；镇痛药；三维结构活性关系

目的：建立μ阿片受体的结构模型，并结合模型研究羟甲芬太尼对该受体的作用机制。**方法**：以细菌视紫红质的三维结构为模板，在计算机上建立μ阿片受体模型，然后将羟甲芬太尼对接到假想的受体结合部位。**结果**：得到了良好的配体-受体相互作用模型，发现残基 Asp147 与 His319 为可能的结合位点。配体质子化 N 原子与 Asp147 形成强的静电和氢键相互作用，羰基 O 原子与 His319 形成弱的静电和氢键相互作用，两个苯环分别与周围的芳香残基形成 π-π 相互作用。**结论**：该配体-受体相互作用模型有助于合理设计新型镇痛药。