ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Feb; 20 (2): 131 - 136
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Molecular modeling on kappa opioid receptor and its interaction with nonpeptide kappa opioid agonists[1]

LIU Dong-Xiang, JIANG Hua-Liang, SHEN Jing-Shan, ZHU Wei-Liang, ZHAO Lei, CHEN Kai-Xian[2], JI Ru-Yun

(*Shanghai Institutes of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS molecular models; pharmacophore map; kappa opioid receptors; analgesics; ligands; cell surface receptors; structure-activity relationship; bacteriorhodopsin

ABSTRACT

AIM: To study the interaction between κ-opioid receptor and its nonpeptide agonists. **METHODS**: The "conservation patterns" for G-protein coupled receptors (GPCR) were used to determine 7 transmembrane (TM) regions. Taking the crystallographic coordinates of bacteriorhodopsin (BR) as the template, the 3D structural model was constructed for 7 TM of κ-opioid subtype with molecular mechanics (MM) method. Five highly active nonpeptide κ-opioid agonists were docked into the 7 helices of κ-opioid receptor to study the ligand-receptor interaction. **RESULTS**: Four important interactions between U-50488-like agonists and κ-opioid receptors were drawn according to our modeling study: (1) the protonated pyrrolidine nitrogen of the ligands formed a hydrogen-bond with the carboxyl of Asp138; (2) the carbonyl oxygen of ligands forms a hydrogen bond to the hydroxyl of Ser187; (3) the aryl groups connected to acylamide of the agonists inserted into a hydrophobic cavity enclosed by residues Val239, Val236, Phe235, Val232, Leu186, and Trp183; (4) the pyrrolidine of the ligands in the complexes was surrounded by Ile290, Asp138, Ile194, Ile135, and Cys131. **CONCLUSION**: The proposed interaction mechanism is helpful for further mutant experiments and designing novel potent κ-opioid agonists.

INTRODUCTION

The discovery that the prototype selective κ-opioid agonists, eg, U-50488, are devoid of respiratory depression, constipations and physical dependence liability side effects associated with morphine-like analgesics suggests that κ-opioid agonists would become one of the most potent analgesic drugs. Since the cloning of the 3 opioid subtypes μ, δ, and κ, a large amount of efforts have been dedicated to the identification of the binding regions in opioid receptors which are responsible for the selectivity. Chimera studies have revealed that three extracellular loops (EL1-EL3) are associated with κ-opioid selectivity for endogenous dynorphin A (dynA)[1]. Site-directed mutagenesis experiments have shown that the 7-transmembrane regions may contain critical residues for binding nonpeptide κ-opioid agonists[2].

Recently, we have established the pharmacophore map of nonpeptide κ-opioid agonists and given some interesting results related to the interaction between nonpeptide κ-opioid agonists and the receptor[3]. For more perspective comprehension on the interaction, we determined to construct the theoretical three-dimensional

[1] Project supported by the National Natural Science Foundation of China (No 29725203) and the special program of Life Science of Chinese Academy of Sciences (No STZ-3-02).
[2] Correspondence to Prof CHEN Kai-Xian.
Phn 86-21-6431-1833. Fax 86-21-6437-0269.
E-mail kxchen@iris3.simm.ac.cn
Received 1998-03-23 Accepted 1998-09-14

ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Feb; 20 (2)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

(3D) model of κ-opioid receptor and investigate the interaction between 5 highly active nonpeptide κ-opioid agonists and the receptor.

METHODS

The crystallographic coordinates of bacteriorhodopsin (BR) were acquired from Protein Data Bank (PDB) (entry 1BRD). According to the hydrophobicity analysis and conservative residues for G-protein coupled receptors (GPCR), residues in 7 transmembrane (TM) regions of κ-opioid receptor corresponding to 7 TM residues of BR were determined (Fig 1). The initial coordinates of the proposed TM residues were taken from BR crystallographic structure.

Then, the residues on 7 TM were assigned Kollmann all-atom charges. Automatic adjustments were made to remove the unfavorable steric interactions caused by sidechains. All sidechains of 7 helices were optimized with conjugate gradient minimization method while the backbone of receptor was aggregated. The overall geometry of 7 helices was optimized until the energy gradient was <0.5 kcal $\cdot$ mol^{-1} $\cdot$ m^{-1}. During the opitimization, Kollmann all-atom force field was applied, the distance-dependent dielectric constant was 5.0, and nonbonded cut-off was set to be 0.8 nm.

To qualify the final receptor structure, we analyzed the values of the dihedral angles of the peptide backbone and side chains (Fig 2). All residues were found in allowed regions of the Ramachandran plot with side chain dihedrals in ideal regions.

Five highly active nonpeptide κ-opioid agonists (MDL167361, GR-89696, MDL163725, MDL158584, and ICI-197067) were chosen to study the interaction with κ-opioid subtype.

According to site-directed mutagenesis experiments, Asp138 residue is critical for the binding of U-50488. Therefore, the 5 U-50488-like agonists were docked into the Asp138 neighboring putative binding sites manually by the comformations presented in our previous pharmacophore study[3]. After optimizing the geometry of the complexes in Tripos/Sybyl 6.2 environment, the interaction machanism was deduced by examination of the 5 complexes model structures.

RESULTS AND DISCUSSION

Modeling of 7 α-helices of κ-opioid receptor Modeling studies on μ- and δ-opioid receptor subtypes have already been reported[4,5]. For the future study on the selectivity

TM1	mOPRK1	60	V I I T A V Y S V V F V V G L V G N S L V M F 82
	BR	10	W I W L A L G T A L M G L G T L Y F L V K G M 32
TM2	mOPRK1	99	F N L A L A D A L V T T T M P F Q S A V Y 119
	BR	42	F Y A I T T L V P A I A F T M Y L S M L L 62
TM3	mOPRK1	131	C K I V I S I D Y Y N M F T S I F T 148
	BR	79	Y W A R Y A D W L F T T P L L L L D 96
TM4	mOPRK1	183	W L L A S S V G I S A I V L G G 198
	BR	111	L V G A D G I M I G T G L V G A 126
TM5	mOPRK1	228	I C V F V F A F V I P V L I I I V C Y T 247
	BR	137	W W A I S T A A M L Y I L Y V L F F G F 156
TM6	mOPRK1	275	L V L V V V A V F I I C W T P I H L F I L V 296
	BR	170	T F K V L R N V T V V L W S A T P V V W L I 191
TM7	mOPRK1	309	L S S Y Y F C I A L G Y T N S S L N P V L 329
	BR	202	N I E T L L F M V L D V S A K V G F G L I 222

Fig 1. Sequence alignment of 7 TM regions between mOPRK1 and bacteriorhodopsin.

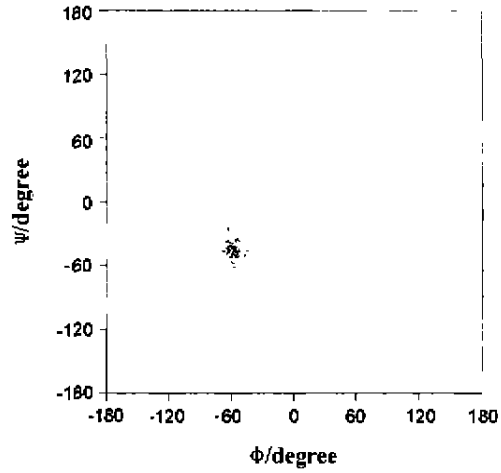
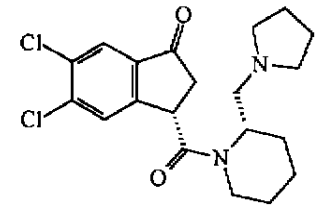


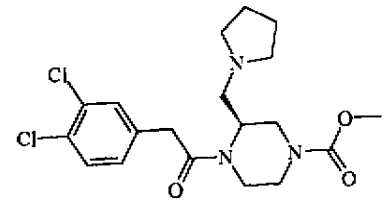
Fig 2. Ramachandran plot of residues on 7 TM.

among the 3 opioid subtypes, we tried to build κ-opioid structure model when no GPCR crystallographic structure is avaliable. Based on the theoretical model, we wished to interpret the interaction between U-50488 like κ-opioid agonists and κ-opioid receptor.

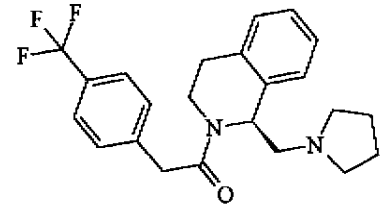
Since the binding sites for U-50488 are located in the region near residue Asp138, construction of the 7 TM regions is adequate to study the interaction. Similar to the previous GPCR modeling, we also employed the crystallographic structure of bacteriorhodopsin as the template. This is because BR has the similar structural and functional characteristics to rhodopsin which belongs to GPCR superfamily even though the primary sequence homology between BR and GPCR is low. But it is still a challenge work to determine the beginning and ending residues, orientation for every TM region. For these purposes, several methodologies were adopted such as Fourier transform methods[6], prediction algorithm of Sander[7]. In present study, we aligned the sequence of κ-opioid receptor on that of other GPCR. The crude segments of the 7 TM were decided by the common aspects of hydrophobicity profiles for many GPCR. Referring to Cappelli's research paper[8], we finally determined the residues on the 7 TM of κ-opioid receptor which are



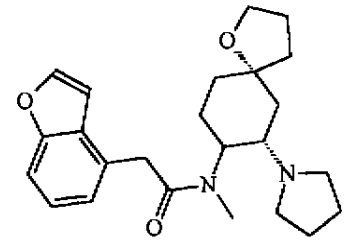
MDL167361



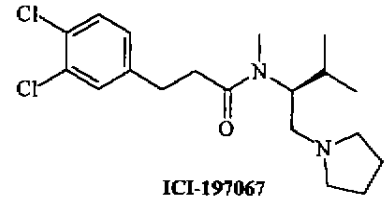
GR-89696



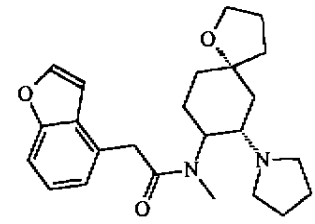
MDL163725



MDL158584



ICI-197067



U-50488

corresponding to the residues on BR 7 TM.

After the sidechain adjustment and the full geometry optimization, the refined structural

ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Feb; 20 (2)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

model was obtained. Most of the hydrophobic residues as Phe, Trp, Val are distributed towards the outside of the receptor. Interestingly, residues with large hydrophobic sidechain as Phe114, Trp183, Phe283, and Trp287 were enclosed in the 7 TM helices. Residue Phe114 was far away from the other three residues. The specific tertiary structure is important for the binding of nonpeptide κ-opioid agonists.

Elucidation of structure-activity relationships of ligands According to the pharmaphore map of nonpeptide κ-opioid agonists, the pyrrolidine, the carbonyl of acylamide and the aryl group attached to acylamide are suggested to be the structure specific moieties of nonpeptide κ-opioid agonists. To verify the pharmaphore map of nonpeptide agonists and to understand the ligand-receptor interaction more deeply, we docked the 5 agonists into the receptor using the pharmacophoric conformations of these ligands.

Because the ligands would display high positive charge at the N atom of pyrrolidine at physiological pH, the atom type of pyrrolidine nitrogen was modified to be N4. The protonated N atom was proposed to hydrogen-bond Asp138 residue. Actually, we found that the ligands could be accomodated within the 7 helices very well. After geometry optimiztion on both receptor and ligand in Dock module of Sybyl 6.2, the binding energies including eletrostatic energy and steric energy (Lennard-Jones 12 – 6 potential) for the 5 complexes were evaluated, which are presented in Tab 1.

Tab 1. Docking energy of ligand-receptor interaction.

Agonist	Energy/kcal·mol^{-1}		
	Steric	Electrostatic	Total
MDL167361	-16.768	-49.131	-66.081
ICI-197067	-30.741	-41.077	-71.818
GR-89696	-34.372	-51.047	-85.369
MDL163725	-31.835	-31.629	-63.463
MDL158584	-25.029	-50.398	-75.428

The low binding energies suggest the good quality of our pharmacophore map and the theoretical model of κ-opioid subtype receptor. For representation, complex structure of protonated ICI-197067 and κ-opioid receptor (α-carbon atom only) is shown in Fig 3.

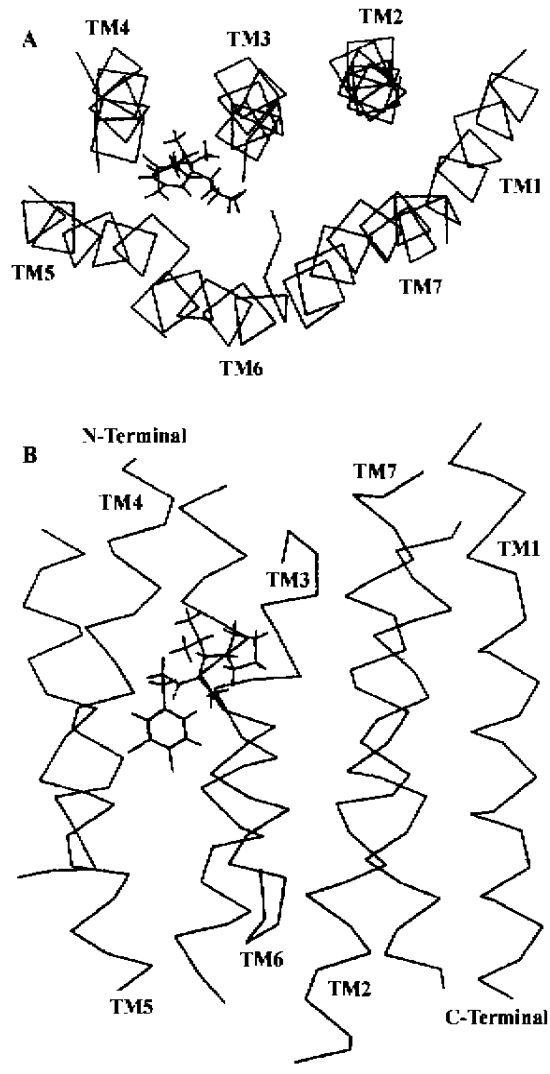


Fig 3. Complex between protonated ICI-197067 and the kappa opioid receptor, shown in α-carbon atom only. (A) View from the top (B) Side view from a direction perpendicular to the main axes of the 7 α-helices.

The detailed view of protonated ICI-197067 interaction with the binding sites of κ-opioid receptor is shown in Fig 4.

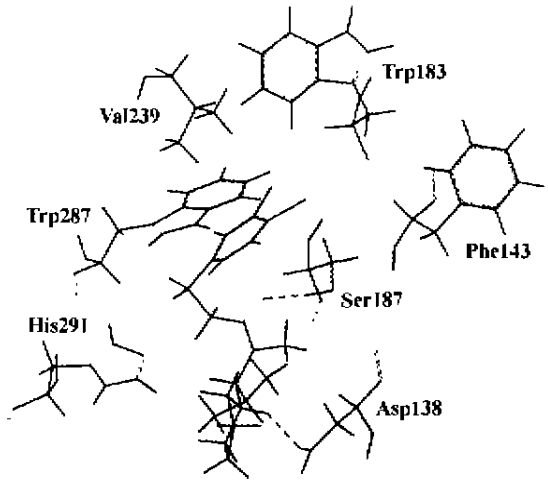
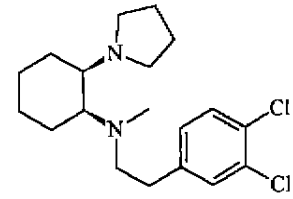


Fig 4. The detailed view of protonated ICI-197067 interaction with the binding sites of κ-opioid receptor.

Some important interaction features can be deduced from the above models. It is obvious that a potent electrostatic and hydrogen-bond interaction occurred between the negatively charged carbonate oxygen of Asp138 and the positively charged N of ligands, which is essential for the κ-opioid selective binding. The pyrrolidine of the ligands in complexes was surrounded by Ile290, Asp138, Ile194, Ile135, and Cys131. The hydrophobic interaction with Ile residue would be the reason why ligand with pyrrolidine has higher affinity than ligands with amine. The distances between the hydrogens of pyrrolidine and the nearest hydrogens of Ile194, Ile135, Cys131 are no more than 0.33 nm. The hydrophobic cavity constituted by these residues is not big enough to accommodate larger groups as phenyl or six-membered ring. Introducing larger group at this position will bring about steric hinderance with the residues. Moreover, the aryl groups connected to the acylamide of ligands would insert into a hydrophobic cavity formed by residues Val239, Val236, Phe235, Val232, Leu186, and Trp183. Replacement of phenyl group by some other rings such as aromatic heterocyclic ring like MDL167361, MDL158584 may also ensure the analgesic activity. Electrophilic groups like Cl on phenyl have significant impact on the binding activity, which suggests that with the aryl group connected to acylamide of agonists would have π-π interaction with the hydrophobic residues. In the previous study, we proposed that Trp287 might interact with the aryl group connected to acylamide of ligands[3]. But our recent modeling study gave another result. Trp287 residue would more likely interact with the phenyl of *N*-substituted tetrahydrogen isoquinoline of MDL163725. In addition, the carbonyl oxygen of the ligands hydrogen-bonded the hydroxyl of Ser187. Some U-50488 like compounds without the carbonate of acylamide do not have the affinity for κ-opioid receptor[9]. Such hydrogen-bond should be the good explanation for the paucity of κ-opioid binding activity due to the vanishing carbonate.



σ-Receptor agonist

GR-89696 is the highest active among the 5 κ-opioid agonists. Its distinctive structure characteristic is the piperazine formic ether. According to our model, the formic ether was enclosed by residues Ser136, Val134, Tyr139, Ile135, and Ile137. The main-chain oxygen of these residues would have beneficial interaction with the formic ether of GR-89696.

From the ligands' orientation in the complexes, some other residues, eg, His291, Gly190, and Ile191, were also included within the sphere of 0.4 nm radius centered the ligands. Although no apparent interaction between these residues and the ligands was revealed, their neighboring positions to the ligands implied that

ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1999 Feb; 20 (2)
E-mail aps@server.shenc.ac.cn Phn/Fax 86-21-6474-2629

they might have influence on the analgesic activity. Therefore, for designing more potent κ-opioid selective agonists, much attention should be paid to the critical residues for the κ-opioid selectivity such as Asp138, Ser187. Besides, introducing more interactions with residues as His291, Gly190, Ile191 would contribute to the binding affinity improvement for κ-opioid receptor.

To conclude, we have generally elucidated the interaction of U-50488 like agonists with κ-opioid subtype and the structure-activity relationships of κ-opioid nonpeptide agonists. These results are helpful for the further mutant experiments aimed to identify the essential residues for κ-opioid selectivity and designing novel selective κ-opioid nonpeptide agonists.

REFERENCES

1 Wang JB, Johnson PS, Wu JM, Wang WF, Uhl GR. Human κ opiate receptor second extracellular loop elevates dynorphin's affinity for human μ/κ chimeras.
J Biol Chem 1994; 269: 25966 - 9.

2 Metzger TG, Paterlini MG, Portoghese PS, Ferguson DM. Application of the message-address concept to the docking of naltrexone and selective natrexone-derived opioid antagonists into opioid receptor models.
Neurochem Res 1996; 21: 1287 - 94.

3 Liu DX, Tang Y, Jiang HL, Chen KX, Ji RY. The Establishment of kappa opioid receptor agonists pharmacophore with molecular modelling method.
Acta Pharmacol Sin 1998; 19: 445 - 50.

4 Tang Y, Chen KX, Jiang HL, Wang ZX, Ji RY, Chi ZQ. Molecular modeling of μ opioid receptor and its interaction with ohmefentanyl.
Acta Pharmacol Sin 1996; 17: 156 - 60.

5 Alkorta I, Loew GH. A 3D model of the δ opioid receptor and ligand-receptor complexes.
Protein Eng 1996; 9: 573 - 83.

6 Donnelly D, Overington JP, Blundell TL. The prediction and orientation of α-helices from sequence alignments: the combined use of environment-dependent substitution tables, Fourier transform methods and helix capping rules.
Protein Eng 1994; 7: 645 - 653.

7 Rost B. Predicting one-dimensional protein structure by profile based neural networks.
Methods Enzymol 1996; 266: 525 - 39.

8 Cappelli A, Anzini M, Vomero S, Menziani MC, De Benedetti PG, Sbacchi M, *et al*. Synthesis, biological evaluation, and quantitative receptor docking simulation of 2-[(acylamino) ethyl]-1, 4-benzodiazepines as novel tifluadom-like ligands with high affinity and selectivity for κ-opioid receptors.
J Med Chem 1996; 39: 860 - 72.

9 De Costa BR, Radesca L, Di Paolo L, Bowen WD. Synthesis, characterization and biological evaluation of a novel class of *N*-(arylethyl)-*N*-alkyl-2-(1-pyrrolidinyl) ethylamines: structural requirements and binding affinity as the σ receptor.
J Med Chem 1992; 35: 38 - 47.

131-136

κ阿片受体分子模拟及其与非肽类κ阿片激动剂的作用[1]

R971.2
R966

刘东祥，蒋华良，沈敬山，朱维良，赵 磊，陈凯先，秘汝运
(中国科学院上海药物研究所，上海 200031，中国)

关键词 分子模型；药效基团；κ阿片受体；镇痛药；配位体；细胞表面受体；结构-活性关系；细菌紫质

目的：研究κ阿片受体及其与非肽类激动剂的作用机制. **方法**：以细菌视紫红质为模板，模建κ阿片受体七个跨膜区的三维结构；将五个高活性非肽类激动剂对接到螺旋区内，研究作用机制. **结果**：(1) 四氢吡咯环氮原子与 Asp138 羧基成氢键；(2) 乙酰胺羰基氧与受体 Ser187 间存在氢键作用；(3) 与乙酰胺相连的疏水基团处于由 Val239、Val236、Phe235、Val232、Leu186 和 Trp183 构成的疏水区域内；(4) 激动剂的四氢吡咯环为 Ile290、Asp138、Ile194、Ile135 和 Cys131 残基包围. **结论**：模型将有助于设计新型高效安全的κ阿片受体激动剂.

(责任编辑 李 颖)