ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8): 701 - 708
http://www.chinaphar.com


# Building 3D-structural model of kappa opioid receptor and studying its interaction mechanism with dynorphin A(1 - 8)[1]

WAN Xu-Hu, HUANG Xiao-Qin, ZHOU De-He[2], JIANG Hua-Liang, CHEN Kai-Xian, CHI Zhi-Qiang
(*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

**KEY WORDS** molecular dynamics simulation; kappa opioid receptors; molecular models; binding sites; dynorphins

## ABSTRACT

**AIM**: To construct the 3D-structural model of human kappa opioid receptor (HKOR) and study its interacting mechanism with dynorphin A(1 - 8) (Dyn8). **METHODS**: Comparative molecular modeling was applied to build the 7 transmembrane (TM) helical domain of HKOR using the bovine rhodopsin (OPSD) model as a template. Molecular dynamics was performed to minimize the HKOR model and to simulate the 3D-structure of Dyn8 based on the NMR results of dynorphin A(1 - 14). The extracellular loops (EL) were built by self-constructed database searching. DOCK4.0 program was performed to construct Dyn8 complex with HKOR. **RESULTS**: (1) The model of HKOR was obtained and validated by theoretical and experimental data. (2) The Dyn8-HKOR interacting mechanism is reasonably explained: Side chain of residue Asp138 interacts with protonated nitrogen atom at the N-terminal residues of Dyn8 through electrostatic and hydrogen bonding, which play an important role in ligand binding with receptor. (3) Negatively charged amino acids in the second extracelluar loop (EL2) as Asp223 and Glu209 interact with the C-terminal positively charged residues in Dyn8, and Glu209 is a likely determinant of peptide ligand specificity. **CONCLUSION**: Some amino acid residues positioned in EL2, TM3, TM4, and TM5 form the binding site and therefore determine the selectivity of kappa peptide agonist.

[1] Project supported by the National Natural Science Foundation of China (No 39630350) and the Ministry of Science & Technology of China (G1999054002) and Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences.
[2] Correspondence to Prof ZHOU De-He.
Phn 86-21-6431-1833, ext 509. Fax 86-21-6437-0269.
E-mail dhzhou@mail.shcnc.ac.cn
Received 2000-01-24 Accepted 2000-04-18

## INTRODUCTION

Dynorphin A is the endogenous selective agonist of human kappa opioid receptor(HKOR)[1], and it shares a common sequence with other endogenous opioid ligands amongst the first four amino acids: Tyr1-Gly2-Gly3-Phe4/Leu4. These residues have been recognized as a message domain which is the shortest fragment that has the binding affinity and does less to the selectivity of ligand[2]. Chimeric peptides study[3] suggests that dynorphin A(6 - 8) (Arg6-Arg7-Ile8) contributes to the biological selectivity by interacting with HKOR and enhances its potency. Chimeric receptor research[4] shows that kappa opioid receptor utilizes its middle domain which comprises the 4th transmembrane helix (TM4) and the highly negatively charged second extracellular domain to achieve its high affinity binding with the prodynorphin peptides. The interaction of a small ligand with the receptor is very different from that of a peptide ligand. The binding of a peptide ligand is more dependent on the combination of specific receptor segments than the binding of a small ligand.

Given the importance of kappa EL2 in DynA binding, we investigated the secondary structure of this region and its relation to both selectivity and affinity. An understanding of the kappa selectivity of DynA may be obtained by considering the properties of this peptide. Both Dyn A (1 - 17) and Dyn A (1 - 14) are partly helical when bound to neutral lecithin membranes[5] or micelles[6]. So we investigated the possibility that the selectivity and affinity of Dyn A was imparted by specific structural elements of EL2 of the HKOR which may interact with the helical portion of Dyn A at the water-membrane interface.

In order to gain a better understanding of the molecular interactions between the HKOR and Dyn A, we have modeled the receptor and its complex with a shorter fragment of Dyn A, Dyn A (1 - 8) (Dyn8). The functional roles of the EL2 domain and the conserved N-terminal tetrapeptide opioid 'message' binding site are dis-

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

cussed. The model provides a basis for further experimental investigation of the interplay of structure and biological functions in this important receptor.

## METHODS

**Modeling of HKOR transmembrane helix** In the absence of high resolution crysal structure for the G-protein coupled receptor superfamily, the bovin rhodopsin (OPSD) model[7] was used as a template to construct the seven-helix transmembrane domain of HKOR. Assignment of the seven TM helix regions was based on primary sequence comparison between KOPR and OPSD along with hydrophobic analysis. An initial model of KOPR was constructed by superposing helices onto $C_\alpha$ coordinates of the template[8] according to the alignment with OPSD. The torsions of HKOR were measured and modified with the normal $\phi$ and $\psi$ values of $-58°$ and $-47°$. Then the values of torsion and hydrogen bond length were constrained.

***Molecular dynamics study of HKOR and* Dyn A(1 – 8)** The initial structure of HKOR was computed with the molecular dynamics (MD) simulation. The parameters of the simulation were chosen as follows: Non-bonded cut-off radius was 8.0; the time step was 1fs; the dielectric constant was 8.0. The tripos force field and kollman-all-atom charge were applied. Then the averaged conformation of the simulation was optimized with the following parameters: a distance-dependent dielectric constant of 8.8, conjugate gradient minimization until the energy gradient RMS < 0.05 kcal (mol ·A)$^{-1}$.

The starting conformation for Dyn A(1 – 8) was taken from the two-dimensional NMR data reported by Tessmer and Kallick and the structure was elucidated using molecular dynamic simulation. The last ten conformations of Dyn A(1 – 8) were averaged and optimized with the above method.

**Modeling the extracellular loops of HKOR** The three extracellular loops connecting the minimized TM helices were then added using loop search command embedded in the homology module of insight Ⅱ. The conserved disulfide bridge connecting Cys131 and Cys210 was built manually. The whole receptor was then optimized using the method described above. Some hydrogen bonds formed by residues from TM helixes and extracellular loops in HKOR were analyzed.

**Docking the Dyn A(1 – 8) to the binding site of HKOR** According to site-directed mutagenesis and sequence analysis, residue Asp138 plays an important role in binding[9]. In addition, EL2 is negatively charged while Dyn A(1 – 8) has two positively charged side chains, recognition of Dyn A(1 – 8) by HKOR would then have occurred through the interaction between residues 6 – 8 of Dyn A(1 – 8) and the second extra cellular loop of HKOR. According to this information, the docking mode was then constructed for subsequent energy minimization.

The whole work was performed on Silicon Graphics IRIS R4000 workstation using Sybyl6.5[14], Insight Ⅱ[13] software package and Dock 4.0.

## RESULTS AND DISCUSSION

**General features of 3D-structural model** The aligned sequence of the modeled transmembrane helices of the HKOR is shown in Fig 1, along with that of OPSD. The seven TM helices of the last model are arranged sequentially and anti-clockwise when viewed from the extracellular side. The orientation of TM helices was in good accordance with those of OPSD.

**Validation of the 3D-structural model of HKOR** The validation of the receptor model is based on the fact that all GPCRs share significant structural commonalities. This leads to the inference that the model must incorporate structural features deduced from studies on a large variety of different receptors. The root mean square deviation (RMSD) over the $C_\alpha$ positions comprising the TM helix framework of the receptor is 1.97 Å compared with the template, and 1.90 Å, for the receptor complexed with Dyn8. During the simulations the α-helical contents of the receptor model increase only slightly from 154 to 162 reflecting the balance between the stability of the helices and the structural dynamics during the simulation.

The reliability of a model is improved considerably if experimental data on the structures are taken into account while devising it. Sites that are important with respect to structure function or ligand binding (as determined by site directed mutagenesis) are expected to face inwards and form a potential ligand binding site. Mutated residue positions in HKOR or other opioid receptors yielding significant changes[10] to binding properties are highlighted in Fig 2.

**Stability of the model** The procedure described in methods for construction of the kappa model results in a molecular structure that was quite stable during MD

| | | | | |
|---|---|---|---|---|
| TM1 | OPSD | 38 | SMLAAYMFLLIMLGFPINFLTLYVTV | 63 |
| | HKOR | 60 | VIITAVYSVVFVVGLVGNSLVMFVII | 85 |
| TM2 | OPSD | 70 | TPLNYILLNLAVADLFMVFGGFTTTL | 95 |
| | HKOR | 92 | TATNIYIFNLALADALVTTTMPFQST | 117 |
| TM3 | OPSD | 111 | NLEGFFATLGGEIALWSLVVLAIERYVVVC | 140 |
| | HKOR | 132 | KIVISIDYYNMFTSIFTLCTMSVDRYIAVC | 161 |
| TM4 | OPSD | 151 | NHAIMGVAFTWVMALACAAPPLVGWS | 176 |
| | HKOR | 173 | LKAKIINICIWLLSSSVGISAIVLGG | 198 |
| TM5 | OPSD | 202 | SFVIYMFVVHFIIPLIVIFFCYGQLVFTVK | 231 |
| | HKOR | 225 | FMKICVFIFAFVIPVLIIIVCYTLMILRLK | 254 |
| TM6 | OPSD | 250 | VTRMVIIMVIAFLICWLPYAGVAFYI | 275 |
| | HKOR | 272 | ITRLVLVVVAVFVVCWTPIHIFILVE | 297 |
| TM7 | OPSD | 286 | IFMTIPAFFAKTSAVYNPVIYIMMNK | 311 |
| | HKOR | 310 | SSYYFCIALGYTNSSLNPILYAFLDE | 335 |
| EL1 | HKOR | 118 | VYLMNSWPFGDVLC | 131 |
| EL2 | HKOR | 199 | TKVREDVDVIECSLQFPDDDYSWWDL | 224 |
| EL3 | HKOR | 298 | ALGSTSHSTAAL | 309 |

**Fig 1. Definitions of transmembrane helices. Alignment of HKOR amino acid sequence with that of OPSD. Consecutive numbering systems for the individual receptors are denoted. Conserved residues are in bold typeface. The (EL1-3) extra-cellular loop regions delimited by the seven transmembrane helices are named.**

simulation. During the simulation, the distance between the $C_\alpha$ atoms of high conservative residues of HKOR changed little (Tab 1).

**Tab 1. Distance between the $C_\alpha$ atoms of highly conservative residues of kappa opioid receptor. The shortest distance change between $C_\alpha$ atoms of kappa opioid receptor, structures extracted from the receptor model MD simulation: $HKOR_0$, 0 ps; $HKOR_{10}$, 10 ps; $HKOR_{20}$, 20 ps; $HKOR_{30}$, 30 ps; $HKOR_{40}$, 40 ps.**

| | Leu149/ Asp138 | Val280/ Pro289 | Asp138/ Tyr330 | Val280/ Phe235 | Trp183/ Asp138 |
|---|---|---|---|---|---|
| $HKOR_0$ | 16.05 | 14.41 | 22.09 | 13.82 | 18.08 |
| $HKOR_{10}$ | 16.01 | 14.61 | 22.25 | 13.86 | 18.04 |
| $HKOR_{20}$ | 15.68 | 14.43 | 22.27 | 13.74 | 17.99 |
| $HKOR_{30}$ | 16.35 | 14.56 | 22.18 | 13.78 | 18.14 |
| $HKOR_{40}$ | 16.12 | 14.38 | 22.16 | 13.94 | 18.01 |

By the end of the simulation, the root mean square deviation (RMSD) of all the $C_\alpha$ atoms of each conforma-

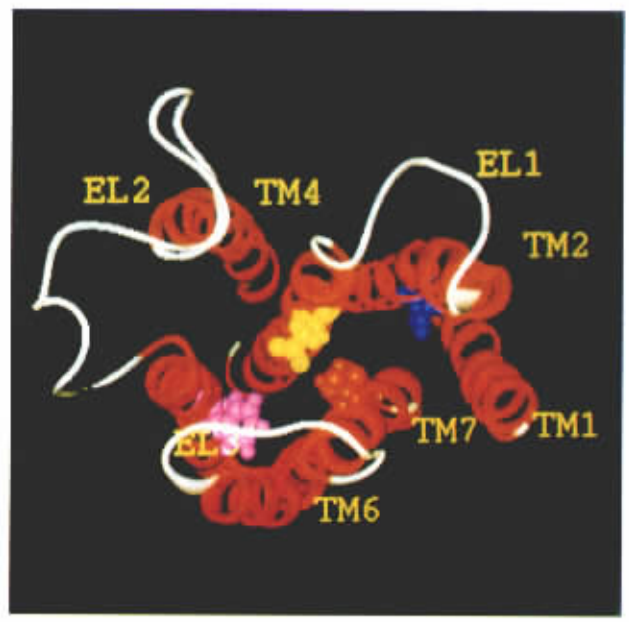


**Fig 2. Top view of the HKOR transmembrane helical bundle from the extracellular surface. Sidechains of residues inferred from single point site directed mutagenesis data are shown in heavy typeface.**

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

tion varied little from the first conformation (Fig 3). The total energy of the model during the MD simulation was found to drop significantly but reached a constant at around 15 ps (Fig 4).

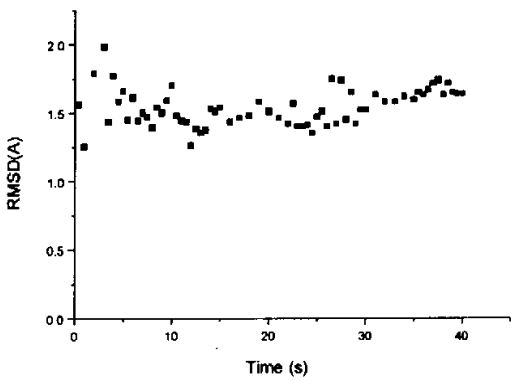


**Fig 3. Root mean square deviation (RMSD) comparision of structures in the MD simulation trajectory of HKOR model. The trajectory shown consists of structures sampled every 0.5 ps over a length of 40 ps. The RMSD considerd only the $C_\alpha$ atoms of the backbone.**

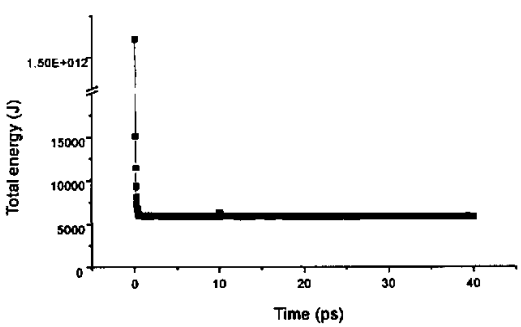


**Fig 4. The total energy changes of the structures of HKOR during the MD simulation. The trajectory shown consists of structures sampled every 50 fs over a length of 40 ps.**

To the 7 TM helices of the minimized receptor were added three extra-cellular loops on the basis of mutual conformational compatibility. Poor steric contacts between loops were relieved by manual tweaking of main-chain and sidechain torsion angles. The final structure (Fig 5) was generated after whole molecule minimization. The H-Bonds of sidechains and loops were checked (Tab 2), these H-Bonds stabilized the structure of the model. The crossing angles between helices of final model and OPSD were compared which showed that almost all the crossing angles were the same except the angle between helix4 and helix5 (Tab 3) suggesting that helix4 and helix5 may be important to the binding of kappa ligand.

**Tab 2. Some hydrogen bonds (besides the backbone hydrogen bonds) formed by residues from TM helices and extracellular loops in HKOR model.**

| Location | Donor | | Acceptor | |
|---|---|---|---|---|
| | Residue | Group | Group | Residue |
| Loop1 | Gly127 | – NH | O = C < | Pro115 |
| | Ser223 | – NH | O = C < | Met121 |
| | Leu120 | – NH | $(-)O^{\delta2}$ – | Gly122 |
| Loop2 | Ser223 | – NH | O = C < | Met121 |
| | Gln213 | – $N^{\varepsilon2}H$ | O = C < | Val207 |
| | Gln213 | – $N^{\varepsilon2}H$ | $(-)O^{\varepsilon2}$ – | Glu209 |
| | Ile208 | – NH | O = C < | Val205 |
| | Val207 | – NH | O = C < | Val205 |
| | Val201 | – NH | O = C < | Glu209 |
| | Glu209 | – NH | O = C < | Val201 |
| | Asp216 | – NH | O = C < | Gln213 |
| | Tyr219 | – NH | O = C < | Asp217 |
| | Asp218 | – NH | $(-)O^{\delta2}$ – | Asp217 |
| | Trp222 | – $N^{\varepsilon1}H$ | $(-)O^{\delta2}$ – | Asp217 |
| Loop3 | Ala308 | – NH | O = C < | Thr306 |
| | Ser310 | – NH | O = C < | Ala308 |
| | Ser303 | – NH | O = C < | ser301 |
| | His304 | – NH | O = C < | Thr302 |
| Helix7 – 7 | Ser311 | – NH | – OH | Ser310 |
| Helix7 – 7 | His304 | – $N^{\varepsilon1}H$ | – OH | Tyr312 |
| Helix5 – 6 | Lys227 | – $N^{\zeta}H$ | $(-)O^{\varepsilon2}H$ | Glu294 |
| Helix3 – 5 | Arg252 | – $N^{\eta2}H$ | O = C < | Ile158 |
| Helix3 – 5 | Arg252 | – $N^{\eta2}H$ | O = C < | Ile158 |
| Helix3 – 3 | Arg156 | – $N^{\varepsilon1}H$ | $(-)O^{\delta1}$ – | Asp155 |
| Helix3 – 3 | Arg156 | – $N^{\zeta}H$ | $(-)O^{\delta1}$ – | Asp155 |
| Helix3 – 3 | Ser136 | – OH | O = C < | Lys132 |

**Tab 3. Crossing angles between helics of kappa opioid receptors and bovine rhodopsin The helical axis was defined by defining the centroid of helix. HKOR: the receptor model non-complexed with Dyn8; HKORd: the receptor model complexed with Dyn8.**

| | Helix1 – 7 | Helix2 – 3 | Helix2 – 7 | Helix3 – 4 | Helix3 – 7 | Helix4 – 5 | Helix5 – 6 | Helix6 – 7 | Helix1 – 2 |
|---|---|---|---|---|---|---|---|---|---|
| OPSD | 40.71 | 151.76 | 135.48 | 141.44 | 44.36 | 162.55 | 142.28 | 168.24 | 146.30 |
| HKOR | 42.10 | 150.58 | 128.10 | 137.05 | 47.78 | 137.05 | 144.39 | 157.67 | 147.89 |
| HKORd | 22.03 | 145.39 | 147.99 | 156.78 | 31.35 | 98.68 | 160.22 | 119.74 | 153.25 |

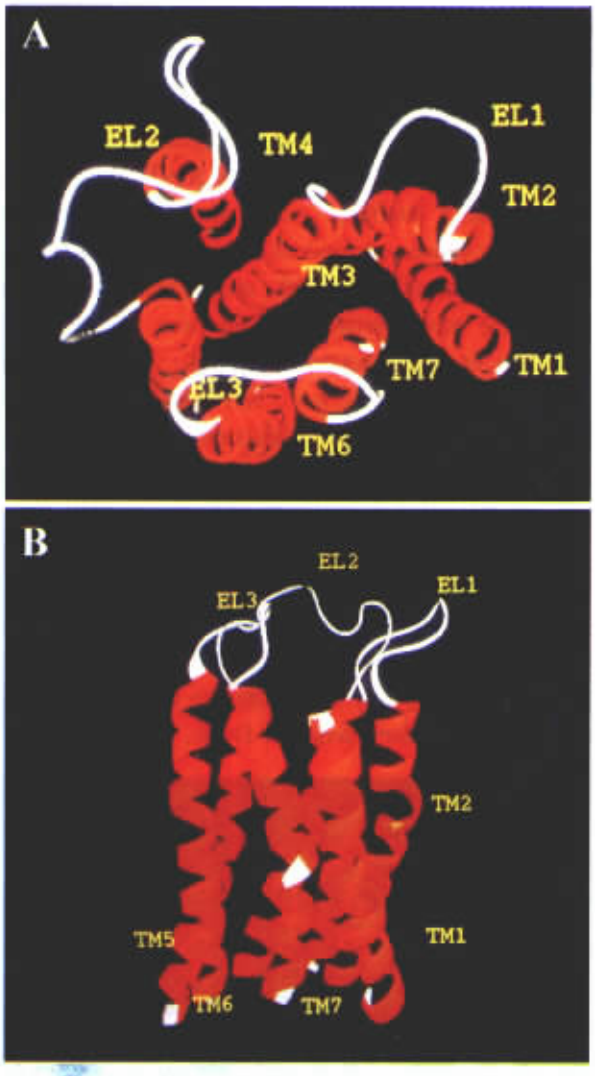


**Fig 5. Cartoon representation of transmembrane helices and extracellular loops of HKOR model. Top view (A) and side view (B) from the extracellular surface are shown above. The TM helices are represented as solid ribbons, and interconnecting loops as tubes.**

**3D-structure of dynorphin A(1 – 8)** Tessmer and Kallick reported that the two-dimensional NMR data of dynorphin A (1 – 17) contained an α helix from residues of Phe4 through Pro10 and a β-turn from residues Trp14 through Glu17. According to this, we constructed the initial backbone of Dyn8 and performed the molecular dynamics simulation. The results suggested that Dyn8 formed partly an α helix from Phe4 to Ile8. After minimization, we got the final structure of Dyn8 (Fig 6), containing four hydrogen bonds in the molecule: Tyr1 C = O···HN Phe4; Phe4 C = O···HN Ile8; Gly3 C = O···HN Arg7; Gly3 C = O···HN Arg6.

**Interacting mechanism of Dyn8 with HKOR** Inspection of the HKOR model revealed that the presence of two hydrophobic pockets in a cavity formed by TM3, 5, 6, and 7, corresponded to the conserved transmembrane opioid binding sites mapped in the delta and mu receptors[11,12]. Both pockets are conserved in the HKOR. Dynorphin A(1 – 4) can be assumed to bind in this binding pocket of HKOR with the sidechains of Tyr1 and Phe4 occuping the two aromatic pockets. The Dyn8:Tyr1 has been docked into the deeper lying pocket, bounded by Ile135, Tyr139, Asp138, Met142, Phe143, Phe231, Val230, Phe225, Ile194, Gly197 (Tab 4). All these residues except Gly197 and Ile194 are conserved in the opioid receptor family. Besides, Tyrosine formed hydrogen bonds with Asp138 and Ile135 (Tab 5).

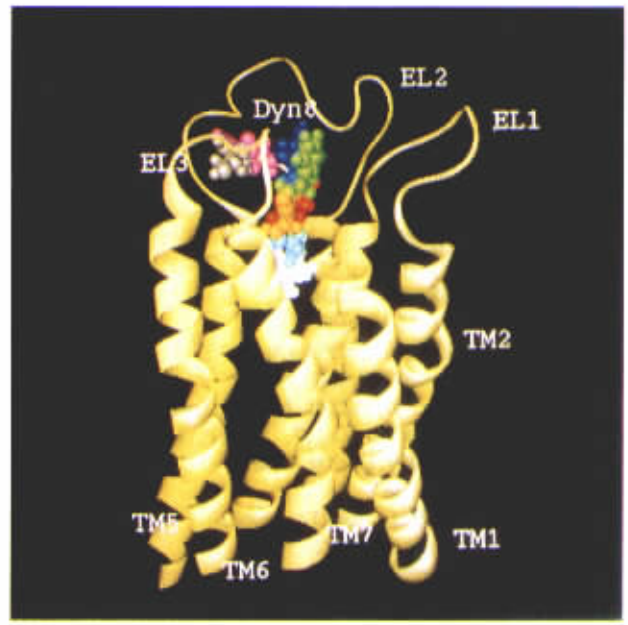


**Fig 6. Dynorphin A(1 – 8)-HKOR complex. The tube representation of Dyn8 is shown bound to the receptor. TM helices are represented as shaded solid ribbon, and the interconnecting loops as tubes. The binding site principally comprises elements from TM helices 3 and 5 – 7, that accommodate the N-terminal (1 – 4) portion of Dyn8, and the acidic second extra-cellular loop with which the positively charged C-terminal of Dyn8 interacts.**

Gly2 and Gly3 of Dyn8 packed close to a number of conserved residues in TM3, 5 and 7. And Gly2, Gly3 formed a hydrogen bond with Asp138 and Glu293 respectively. An intra-molecular hydrogen bond (Gly3 – NH ···O = C < Phe1) further stabilized the Dyn8 conformation in this region. The Phe4 sidechain of Dyn8 located in the adjacent hydrophobic pocket closer to the extracellular surface. This pocket again comprised of several conserved (Ile148, Tyr312) residues. The crossing

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

**Tab 4. HKOR residues within 5 Å of Dyn8 in the energy minimized model complex. The residues within 5 Å of Dyn8 are shown below. The regions where these residues located were also denoted. The highly conserved resides are in bold typeface.**

| Dyn8 residues | HKOR structural element | HKOR residue |
|---|---|---|
| Tyr1 | TM3 | **Ile135, Asp138, Tyr139, Val134, Phe143, Ile146** |
| | TM4 | IlE194, Gly197 |
| | TM5 | **Phe231, Val230, Lys227, Phe235, Ala234** |
| Gly2 | TM3 | **Leu135, Val134, Asp138, Tyr139** |
| | TM5 | **Lys227, Phe231** |
| | TM6 | Glu297 |
| | TM7 | Tyr312, Ile316 |
| Gly3 | TM3 | **Leu130, Asp138, Val134** |
| | TM5 | **Lys227, Phe231** |
| | TM6 | Glu297 |
| | TM7 | Tyr312, Ile316 |
| Phe4 | TM3 | **Ile135, Val134** |
| | EL1 | **Cys131, Leu130** |
| | EL2 | Cys210, Ser211, Leu212 |
| | TM5 | **Lys227** |
| | TM7 | Tyr312 |
| | EL3 | Thr302, His304 |
| Leu5 | TM3 | **Leu130, Ile135** |
| | TM4 | Leu196, Gly197, Gly198 |
| | El2 | **Lys200, Cys210, Ser211** |
| | TM5 | Phe225, Val230, Met226, Lys227 |
| Arg6 | El2 | **Arg202**, Val207, Ser211, Leu212, Gln213, Tyr219, Glu209, Cys210 |
| | TM5 | Met226 |
| Arg7 | El2 | Ser211, Asp217, Asp218, Trp221, Trp222, Asp223, Leu224 |
| | TM5 | Phe225, Met226 |
| Ile8 | EL2 | Ser220, Asp223 |
| | TM5 | Met226 |
| | TM6 | Glu297 |
| | TM7 | Tyr312 |
| | EL3 | His304, Ser303, Ala298, Gly300, Leu299 |

**Tab 5. The hydrogen bonds formed between the Dyn8 and HKOR are denoted below. The bond length is also defined.**

| Residue of Dyn8 | Residue group | Residue group | Residue of HKOR | Elements of HKOR | Bond length |
|---|---|---|---|---|---|
| Tyr1 | –NH | O=C< | Ile135 | TM3 | 2.49 |
| Tyr1 | –NH | $(-)$O(2– | Asp138 | TM3 | 1.932 |
| Gly2 | –NH | $(-)$O(2– | Asp138 | TM3 | 1.947 |
| Gly3 | –NH | $(-)$O(2– | Glu297 | TM6 | 2.535 |
| Arg6 | $-N^{\varepsilon 1}H$ | $(-)O^{\varepsilon 2}-$ | Glu209 | EL2 | 1.824 |
| Arg6 | $-N^{\varepsilon}H$ | $(-)O^{\varepsilon 2}-$ | Glu209 | EL2 | 2.238 |
| Arg7 | $-N^{\varepsilon}H$ | $(-)O^{\delta 2}-$ | Asp223 | EL2 | 1.771 |
| Ile8 | –OH | O=C< | Ser220 | EL2 | 1.948 |

angles between helices of model complexing with Dyn8 and OPSD changed especially the one at helix4 and helix5 (Tab 3), which strengthed the idea that helix4 and helix5 contributed most to the binding of ligand.

Dynorphin A(6–8) binded at the EL2 loop-transmembrane helix interface. In contrast to pockets, the only entirely conserved residues within 5A of dynorphin A (6–8) were Arg202, Cys210, and Ala298. Prominent amongst the most variable residues was Glu209 which formed the hydrogen bound with Arg6. The respective equivalent residues in the mu-, and delta-opioid receptors were Thr220 and Met199. Structure-activity studies using dynorphin A/nociceptin chimeric peptide showed Arg6 was the major determinant of kappa opioid receptor selectivity. So the Glu209 of HKOR was thought to be a likely determinant of HKOR specificity.

The positively charged dynorphin A(6–8) core mainly interacted with the acidic EL2 loop (Fig 6, 7), this interaction not only contributed to the selectivity but also strengthened the potency of Dyn8.

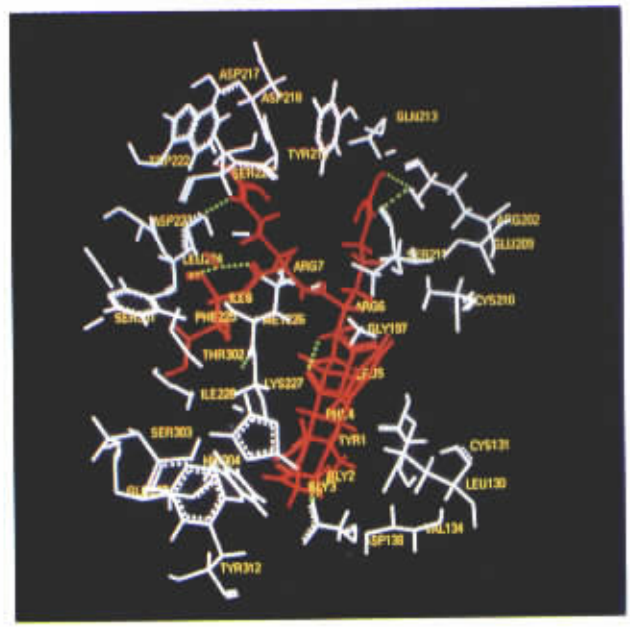


**Fig 7. Binding of dynorphin A(1–8) to the HKOR. The view shows the interaction of dyn8, colored according to the atom type (C, black; H, blue; O, red), with the extracellular termini of TM helices receptor.**

**Possible interaction mode of dynorphin A(1–9) and dynorphin A(1–17) with HKOR**

The predicted docking arrangement of Dyn A(1–9) and dynorphin A(1–17) also placed the opioid "message" of Dyn A(YGGF) within the cavity of the receptor. The

fragment formed an ion pair with Asp138 in TM3, which had been implicated in previous binding studies which showed a loss in affinity following either a D138A or a D138N mutation. The phenyl ring of Tyr1 was also directed to a region rich in aromatic residues that was fairly conserved across the opioid receptor types.

*In kappa opioid receptor, removal of as many as* 10 residues from the C-terminus of dynorphin A has been shown to exert a limited effect on binding and activity. Moreover the chimeric peptide of dynorphin A(1 – 17) in which 9 residues from the C-terminus have been replaced by those of nociceptin, exhibited essentially the same binding and activity characteristics as dynorphin A towards the kappa opioid receptor. So the nine residues of the C-terminus of Dyn A(1 – 17) might not form strong interaction with the EL2. According to the complex of Dyn8 and HKOR, Arg9 could further form hydrogen bonding with the Glu203 or Asp206.

To conclude, the N terminal YGGF tetrapeptide of dynorphin A proposed to bind in a highly conserved transmembrane region. Dynorphin A(5 – 8) binded at the receptor (EL2) loop-transmembrane helix interface in a largely nonconserved region. It may be supposed that interaction of the YGGF with transmembrane opioid binding pocket was crucial for binding affinity, and Arg6 – Arg7 were responsible for the selective preference of the kappa opioid receptor for dynorphin A. These results are helpful for the further mutant experiments aimed to identify the key residues for selectivity and for designing novel selective kappa opioid peptide agonists.

## REFERENCES

1 Chavkin C, Goldstein A. Demonstration of a specific dynorphin receptor in guinea pig ileum myenteric plexus. Nature 1981; 291: 591 – 3.

2 Goldstein A, Fischli W, Lowney LI, Hunkapiller M, Hood L. Porcine pituitary dynorphin: complete amino acid sequence of the biologically active heptadeca peptide. Proc Natl Acad Sci USA 1981; 78: 7219 – 23.

3 Sphie L, Christiane M, Honore M, Gilles C, Catherine M, Jean-Claude M. Comparison of the structure-activity relationships of nociceptin and dynorphin A using chimeric peptides. FEBS Letters 1997; 417: 333 – 6.

4 *Fan M, Mary TH, Robert CT, Larry T, Stanley JW, Huda* A. A chimeric study of the molecular basis of affinity and selectivity of the κ and the δ opioid receptors. J Biol Chem 1995; 270: 12730 – 6.

5 Erne D, Sargent DF, Schwyzer R. Prefered conformation, orientation, and accumulation of dynorphin A-(1 – 13)-tridecapeptide on the surface of neutral lipid membranes. Biochemistry 1985; 24: 4261 – 3.

6 Tessmer MR, Kallick DA. NMR and structural model of dynorphin A-(1 – 17) bound to dodecylphosphocholine micelles. Biochemistry 1997; 36: 1971 – 81.

7 Herzyk P, Hubard RJ. Automated method for modeling seven-helix transmembrane receptors from experimental data. Biophys J 1995; 69: 2419 – 42.

8 Herzyk P, Hubard RJ. Automated method for modeling seven-helix transmembrane receptors from experimental data. Biophys J 1995; 69: 2419 – 42.

9 Kong HY, Raynor K, Yasuda K, Moe ST, Partoghese PS, Beu GI, *et al*. A single residue, aspartic acid 95, in the δ opioid receptor specifiies selective high affinity agonist binding. J Biol Chem 1993; 268: 23055 – 8.

10 Evans SV. SETOR: hardware-lighted three-dimensional solid model representations of macromolecules. J Mol Graphics 1993; 11: 134 – 8.

11 Mansour A, Taylor LP, Fine JL, Thompson RC, Hoversten MT, Moskerg HI, *et al*. Key residues defining the mu-opioid receptor binding pocket: a site directed mutagenesis study. J Neurochem 1997; 68: 344 – 53.

12 Befort K, Tabbara L, Kling D, Maigret B, Kieffer B. Role of aromatic transmembrane residues of the δ-opioid receptor in ligand recognition. J Biol Chem 1996; 271: 10161 – 8.

13 Insight Ⅱ: Version97.5. California: Molecular Simulation Inc; 1997.

14 Sybyl: Version6.5. St Louis(MO): Tripos Associates Inc; 1999.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

## κ阿片受体三维结构的模建及其与强啡肽 A(1－8)作用机制的研究[1]

万旭虎，黄小琴，周德和[2]，蒋华良，陈凯先，池志强 （中国科学院上海药物研究所，上海 200031，中国）

**关键词** 分子动力学模拟；κ阿片受体；分子模型；结合位点；强啡肽类

**目的**：模建人 Kappa 阿片受体(HKOR)三维结构，并研究它与强啡肽 A(1－8)(Dyn8)的相互作用机制．**方法**：以牛视紫红质(OPSD)为模板，运用比较分子模拟模建 HKOR 七段跨膜区的三维结构．运用分子动力学优化 HKOR 模型并根据强啡肽 A(1－14)核磁共振结果构建其三维结构，通过自建数据库搜寻建立 HKOR 的膜外环区，应用 DOCK4.0 将强啡肽 A(1－8)与 HKOR 进行对接．**结果**：(1) 得到 HKOR 三维模型，并用理论及实验参数进行了校正．(2) 合理解释了 Dyn8-HKOR 相互作用机制：Asp138 通过与 Dyn8 的 N 端残基形成氢键及静电作用，在配体受体结合过程中起着重要的作用．(3) HKOR 膜外第二环区(EL2)中带负电荷的氨基酸 Asp223 和 Glu209 与 Dyn8 的 C 端带正电荷的残基相互作用，而 Glu209 可能是决定肽类配体特异性的一个重要因素．**结论**：EL2，TM3，TM4，TM5 上的一些关键氨基酸残基决定 Kappa 阿片受体与肽类配体的选择性结合．

（责任编辑 刘俊娥）